"An update on *Salmonella* prevention and control in poultry by vaccination, including emerging monophasic *S. Typhimurium*

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**Introduction**

Around about 1987, the number of human food-borne infections with *Salmonella* increased spectacularly, not only in the different member states of the E.U., but also in other parts of the world, to the point that some scientists considered this a *Salmonella* pandemic. The increase was almost exclusively due to *Salmonella* Enteritidis, a serotype that was almost absent from the records of human outbreaks prior to 1987. These infections were soon traced back to the consumption of fresh table eggs. In response to this threat, the European Commission issued a directive (92/117) in 1992 "for the protection against specified zoonoses and zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxications."
The adopted strategy was a top-down approach, meant to eradicate *Salmonella* Enteritidis infections in breeding flocks, based on the observation that the infection was transmitted vertically in the layer production chain. In spite of these measures, however, the number of human cases continued to rise, to the point that, in 2000, the Scientific Committee on veterinary measures relating to public health formally stated that “measures in place to control food-borne zoonoses are insufficient”. They also concluded that monitoring and collection of epidemiological data needed to be improved.

Following up on this, the European Commission issued a regulation (2160/2003/EC) “to ensure that measures are taken for the detection and control of *Salmonella* at all levels of the production chain” and “to ensure that all member states start a national monitoring and control program. The key element of the new strategy was that certain targets needed to be met within certain deadlines and time limits, specifically for those *Salmonella* types that are considered of public health importance (Enteritidis, Typhimurium, Infantis, Hadar and Virchow).

Regarding the breeders, the target was set at less than 1% of *Salmonella* positive flocks by the end of 2009. Regarding the layers, the target was defined as a minimum percentage of reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium positive flocks, to finally end up with a maximum percentage of 2% positive flocks throughout the E.U.

In order to calculate the efforts that each member state had to make, an E.U. wide survey of *Salmonella* Enteritidis and Typhimurium prevalence was carried out between October 2004 and September 2005. This so-called baseline studies revealed an E.U. average of 30.8% *Salmonella* positive layer flocks. The annual percentage of reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium positive layer flocks to be achieved in each of the member states depended on the level of flock contamination in the member state, as determined in the baseline study.

To reach these objectives, a number of different tools can be used. Among these, hygiene and disinfection, rodent and insect control, as well as good farming management practices are of vital importance. In addition, a special Commission Regulation (1177/2006) has been issued, prohibiting the use of antimicrobials for the control of *Salmonella*. Feed additives and drinking water additives, other than antibiotics, however, can be used for *Salmonella* control.

### Pathogenesis of egg contamination and consequences for vaccination

Many epidemiological studies have come to the conclusion that internal *Salmonella* contamination of table eggs is mostly due to the serotype Enteritidis (and to some extent also Typhimurium). Only a very small fraction of eggs is contaminated internally with other *Salmonella* serotypes. This is in contrast to the laying hens, where other serotypes are found in approximately 50% of cases.

This contamination of the egg contents is important in the epidemiology of food-borne *Salmonella* infections in humans. Indeed, only for serotype Enteritidis there is convincing epidemiological evidence pointing to fresh eggs as the source of infection. *Salmonella* Enteritidis thus appears to be successful in colonizing the internal content of hen’s eggs. Nevertheless, in fertilized eggs, this does not seem to significantly affect the hatchability of the eggs, thus allowing for efficient vertical transmission of the infection.

The mechanism underlying this phenomenon has been partially unraveled. Originally it was thought that *Salmonella* Enteritidis would contaminate the exterior of the egg shell and, after oviposition, as the egg was cooling down, the bacteria would pass through the pores of the shell.

There is, however, no evidence that *Salmonella* Enteritidis would be more capable than any other serotype to pass through the egg shell and shell membranes.

This leaves the possibility of *Salmonella* contaminating the egg contents during formation of the egg. We showed that any *Salmonella* serotype is able to colonize different organs of the hen after intravenous inoculation, whereas serotype Enteritidis (and to some extent also Typhimurium) is more capable than other serotypes to colonize the oviduct of the hen. Moreover, we showed that *Salmonella* Enteritidis (and to some extent also Typhimurium) is much more resistant to the antimicrobial activity of egg white at the body temperature of the hen (42°C). The fact that eggs are contaminated only with 2 serotypes and that this contamination apparently seems to take place inside the body of the hen opens up opportunities for protection by vaccination.
Vaccination of laying hens against *Salmonella*

The final goal of vaccination is to reduce egg contamination. In order to test the effect of laying hen vaccination on egg contamination, we designed a severe challenge model, using intravenous challenge, collecting and analyzing eggs at the end of the bacteremic phase, i.e. week 2 and 3 after intravenous challenge.

We compared control non vaccinated hens with hens receiving a regular vaccination scheme with live attenuated *Salmonella* vaccines, either AviPro® Salmonella Vac E or AviPro® Salmonella Vac T or AviPro® Salmonella Duo. To our surprise, not only AviPro® Salmonella Vac E, but also AviPro® Salmonella Vac T gave a strong protection against egg contamination. Moreover, when eggs were examined from hens vaccinated with AviPro® Salmonella Duo, even in this severe challenge model, none of the eggs collected in week 2 and 3 after challenge were contaminated with *Salmonella*. These and other observations were taken into account in a scientific advice to the European Commission. In Regulation 1177/2006, vaccination against *Salmonella* was made obligatory in laying hen flocks in member states where the *Salmonella* Enteritidis flock contamination was above 10%.

Since the introduction of obligatory vaccination of the laying hens, the number of confirmed human cases of *Salmonellosis* has drastically declined. More recently, we did some epidemiological studies to find an explanation for the limited number of residual persistently infected layer flocks. It turned out that these were farms where hygienic measures were lacking or were not correctly implemented.

The monophasic variant

To cut a long story short, *Salmonella* monophasic variant is actually a *Salmonella* Typhimurium that expresses only one flagellar antigen instead of two. It is thought to originate from aquaculture production sites in South East Asia. It is in focus because of the recent spectacular rise in human cases infected with this serotype, to the point that some scientists consider this as the onset of a new *Salmonella* pandemic. The problem is taken very seriously, because of the antibiotic resistance pattern of these strains.

They are indeed multiresistant and thus are considered ESBL. Originally, these strains were considered to be associated with pigs and pig meat. A recent survey and molecular typing of strains isolated in the Belgian food chain, however, showed that one in five of these strains were from poultry and poultry meat. In order to evaluate the protection afforded by vaccination against the monophasic variant, we carried out an experiment in which we compared non vaccinated chicks with chicks vaccinated at day 1 after hatch with either AviPro® Salmonella Vac T or AviPro® Salmonella Duo, and then challenged at day 14 with a high dose (10^8 cfu) of *Salmonella* monophasic variant. At 14 days after challenge, both groups of vaccinated birds were very well protected against invasive infection (tested by titration of spleen samples, figure 1).

![Figure 1: Quantitative determination of the germ count (monophasic *S. enterica* subsp. enterica Serovar 1,4,[5],12:i:-) in the caecum and spleen 14 days post challenge infection.](image-url)
Conclusions

Taking into account that many different animal species, including many different wild animals, can carry Salmonella, one should consider Salmonella to be part of the ecosystem, and thus complete eradication of Salmonella seems unrealistic. This has been illustrated by the failure of the first E.U. Salmonella control program, which aimed at eradication. In contrast, a strategy using live attenuated vaccines in combination with hygiene and good farming practice appears to be highly rewarding. In the vaccination strategy, the combined use of both live attenuated Salmonella Enteritidis and Typhimurium vaccine appears to confer superior protection. Moreover, these vaccines provide protection also against the new threat of Salmonella monophasic variant.

Bibliography