Controlling salmonella in poultry flocks by vaccination

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Foodborne infections with salmonella serovars S. enteritidis and S. typhimurium are a serious public health concern. After more than a decade of combating salmonella infections, this organism still represents an important cause of human disease.

Recent studies estimate 80.3 million annual cases of foodborne disease related to salmonella worldwide. Within the European Union salmonella is the second most important cause of foodborne infections after campylobacter.

The consumption of poultry meat and eggs, which represent a major source of cheap high energy protein for much of the world, is believed to be the main cause for salmonella infections in humans.

Significant progress

In a joint effort, the poultry meat and egg industry and the authorities have made significant progress in reducing the contamination rate of poultry flocks and products during recent years in Europe. The economic necessities that are connected with the poultry slaughter process make it much more practical to control salmonella on the poultry farm than trying to do that in the slaughterhouse.

In many countries, including the EU, a chemical treatment of table eggs and poultry meat is not allowed making salmonella control on the farm the important intervention point. Vaccination against salmonella is a well established and effective part of the pre-harvest control strategy. Looking at the number of laboratory reports for human salmonellosis cases in the UK, it can be seen that a clear drop could be observed when salmonella vaccination of chickens was introduced.

When vaccination first arose as a method of combating this organism, inactivated vaccines were developed by various companies. Due to many reasons, such as ease of application, animal welfare, and especially efficacy, attenuated live vaccines entered the market with great success a short time later.

Live vaccines produce better protection than killed vaccines due to the fact that they help to prevent organ colonisation as early and as effectively as possible and reduce shedding and spreading of salmonella. Inactivated vaccines have been tested with varying results and only stimulate antibody production.

They may also lead to poor immune protection due to the destruction of relevant antigens during vaccine preparation and the fast destruction and elimination of the vaccine from the inoculated animals.

They can present only those antigens that were induced under the conditions of the fermentation process.

Their protective efficacy is additionally restricted by their low immunogenicity in unprimed hosts and the fact that they do not induce cytotoxic T cells. Furthermore, inactivated vaccines do not elicit secretory IgA responses, which play an important role in protecting mucosal surfaces.

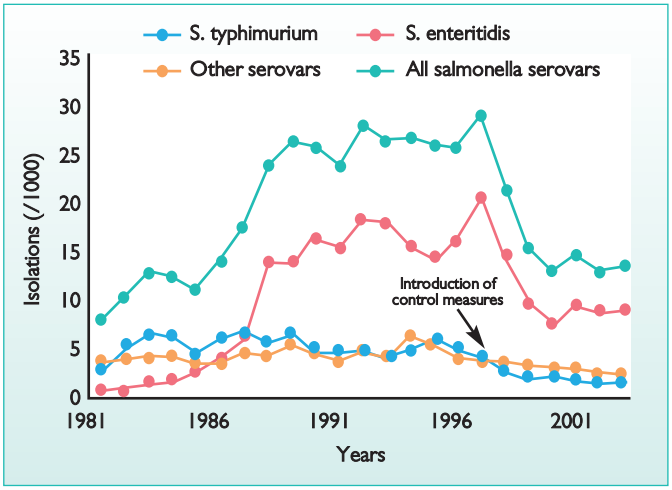


Fig. 2. The number of laboratory reports for human salmonellosis cases in the UK drops after the introduction of salmonella vaccination of chickens (Prof. Paul Barrow, University of Nottingham).

On the other hand, live vaccines reduce the colonisation of the intestine more efficiently. They stimulate a prevailing Th1 (T-cell helper 1) rather than a Th2 (T-cell helper 2) response. The Th1 response is assumed to be important for the elimination of the bacteria from the gut or the tissues. For this reason, live vaccines should always be used in salmonella control, either alone or in combination with inactivated vaccines.

Live vaccines

Live attenuated vaccines derived from S. enteritidis and S. typhimurium are widely used as homologous vaccines against either S. enteritidis or S. typhimurium and their efficacy, ease of use and excellent safety under field conditions has been proven.

The two very effective salmonella live vaccines, AviPro Salmonella Vac E and AviPro Salmonella Vac T, developed by Lohmann Animal Health, have been derived using the metabolic drift mutation (MDM) attenuation technology.

MDM is a naturally occurring process. All microbial populations are genetically heterogenous and

Continued on page 12

Fig. 1. Distribution of food vehicles in strong evidence foodborne outbreaks caused by salmonella in the EU, 2010 (EFSA News, November 2012).

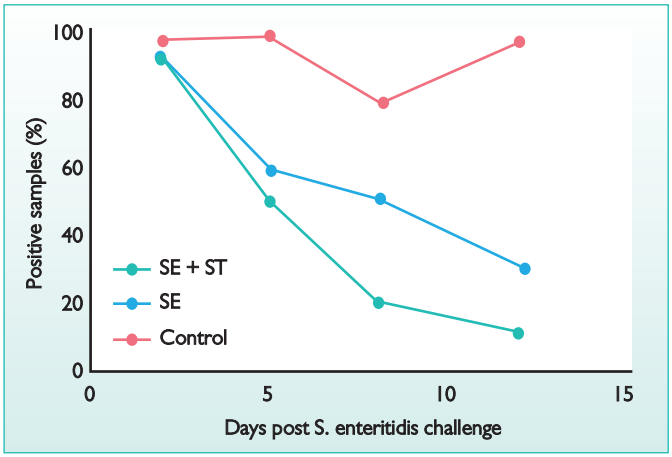


Fig. 3. Protection after challenge in week 29 with S. enteritidis after combined use of AviPro Salmonella Vac E + AviPro Salmonella Vac T.

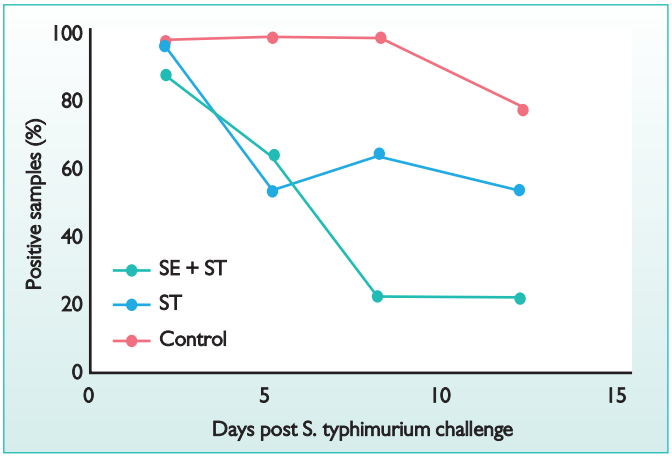


Fig. 4. Protection after challenge in week 29 with S. typhimurium after combined use of AviPro Salmonella Vac E + AviPro Salmonella Vac T.

Continued from page 11
contain 'attenuated mutants' which leads to strains that possess a reduced virulence. The vaccine strains are not genetically-modified and carry minus mutations in essential enzymes and metabolic compartments due to a spontaneous mutation.

The spontaneous mutations are located on the bacterial chromosome. Therefore, they are stable and cannot be transferred via plasmid transfer. As three independent genetic markers are present in each strain, the probability of back mutations is reduced to less than 1:10 exp. 24.

The mutations lead to longer generation times resulting in a decreased and harmless virulence, preserving the immunogenic effect.

At the same time, these strains carry antibiotic markers that allow the diagnostic and differentiation of vaccine and field strains. Therefore, these strains can be used as vaccine strains successfully. The combined use of AviPro Salmonella Vac E and AviPro Salmonella Vac T proved to be completely safe and respective trials proved that protection after challenge with S. enteritidis and S. typhimurium was not influenced when the two vaccines were given together via the drinking water. Shedding and persistence of the challenge strains were significantly reduced in the groups that received both vaccines.

New bivalent live vaccine

Recently, a new bivalent live vaccine consisting of live attenuated S. enteritidis and S. typhimurium strains obtained marketing authorisation in Europe: AviPro Salmonella Duo. Through expertise and vision Lohmann has become the first animal health company to develop a combined homologous salmonella live vaccine for the most important serotypes known today, S. enteritidis and S. typhimurium.

AviPro Salmonella Duo contains the two vaccine strains already successfully used in AviPro Salmonella Vac E and AviPro Salmonella Vac T.

The bivalent vaccine is produced in an innovative co-fermentation process which allows the simultaneous fermentation of both salmonella vaccine strains in one fermenter. In vitro studies provide evidence that there are no inhibitory effects of the two vaccine strains.

Co-fermentation could successfully be upscaled to provide a product that is safe, efficacious and easy to use. Equal titers for both vaccine strains were achieved throughout all steps of the manufacturing process.

This fact ensures the secure maintenance of all their immunogenic properties due to the equal immunogenic effect in the bird for both vaccine strains. The presence of two different vaccine strains in this novel co-fermented live vaccine requires the application of adapted diagnostic procedures to clearly identify both strains in parallel and to distinguish them from salmonella field strains.

The differentiation is based on the antibiotic markers carried by the vaccine strains. The active immunisation with AviPro salmonella Duo effectively reduces the faecal shedding and the colonisation of internal organs with S. enteritidis and S. typhimurium field strains and the S. enteritidis contamination of eggs. In ducks the active immunisation with AviPro Salmonella Duo effectively reduces the colonisation of internal organs with S. typhimurium. In turkeys the colonisation of internal organs with S. enteritidis and S. typhimurium field strains is effectively reduced by the active immunisation with AviPro Salmonella Duo.

Vaccination scheme

The vaccination scheme for chickens consists of three vaccinations during rearing offering long lasting protection – at first day of life, at an age of 6-8 weeks and around the 16th-18th week of life. It is of key importance to vaccinate the birds as early as possible to get an optimum protection.

In turkeys for meat production a single dose from the first day of life followed by a second vaccination at an age of six weeks should be applied. In turkey breeders a single dose from the first day of life followed by a second vaccination at an age of six weeks, a third vaccination at an age of 16 weeks and a fourth vaccination at an age of 23-24 weeks should be applied. Duration of immunity could be shown for 10 weeks after the last vaccination in turkeys for meat production and for at least 30 weeks (SE)/28 weeks (ST) after the last vaccination in turkey breeders, thus covering the whole laying period.

A single dose from the first day of

Fig. 5. Persistence of the S. enteritidis challenge strain in internal organs after infection in week 68 was significantly reduced in the caeca and completely inhibited in the liver after vaccination with AviPro Salmonella Duo.

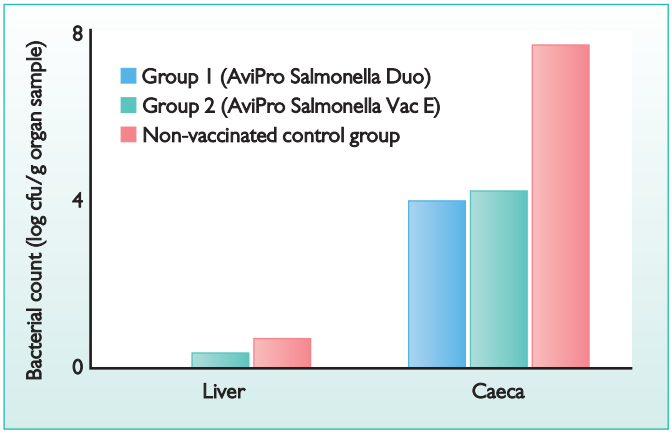
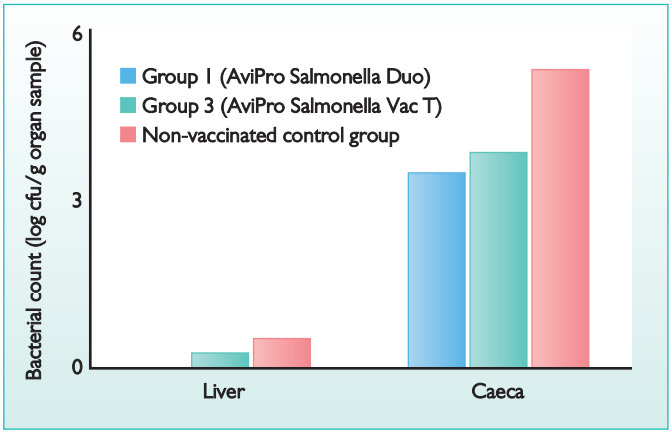


Fig. 6. Persistence of the S. typhimurium challenge strain in internal organs after infection in week 62 was significantly reduced in the caeca and completely inhibited in the liver after vaccination with AviPro Salmonella Duo.



life protects ducks until the end of the fattening period (at least 49 days). Extensive studies demonstrated the safety of AviPro Salmonella Duo in day-old SPF-chicks which are the most susceptible target. No transmission of the vaccine strains on or into eggs could be observed.

The dissemination of the vaccine strains is limited to 21 days for S. enteritidis and 28 days for S. typhimurium.

The S. enteritidis vaccine strain persists in the organs for less than 21 days and the S. typhimurium strain is not detectable within 28 days; this is very important because it leads to a minimal spread to non-target species and a low persistence in the environment.

Efficacy trial

In another trial the efficacy of the vaccine was investigated after oral challenge infection with either S. enteritidis or S. typhimurium field strains. SPF birds were vaccinated with either AviPro Salmonella Duo, AviPro Salmonella Vac E or AviPro Salmonella Vac T, while a fourth group of non-vaccinated SPF birds of the same age was kept as control birds. The birds were infected with high doses of virulent S. enteritidis or S. typhimurium field strains at the beginning or at the end of the laying period.

Seven days after infection liver and caeca samples were analysed bacteriologically (quantitatively) for the presence of the challenge strains. Vaccination with AviPro Salmonella Duo significantly reduced persistence of the challenge strains in liver and caeca in case of the early and late challenges with S. enteritidis or S. typhimurium field strains.
Also in comparison to the birds vaccinated with either AviPro Salmonella Vac E or AviPro Salmonella Vac T a reduced persistence of the challenge strains in liver and caeca could be seen in the group vaccinated with AviPro Salmonella Duo. As a consequence, in case of an infection with S. enteritidis or S. typhimurium field strains a preceding vaccination with AviPro Salmonella Duo can lead to a significant reduction of liver and caeca colonisation, the primary locations of salmonella persistence.
Additional trials proved that the vaccine is not transferred on or into eggs and that the dissemination is limited.

In further trials it could also be shown that a vaccination with AviPro Salmonella Duo prevents the contamination of eggs even under the extreme conditions of an intravenous S. enteritidis challenge.

Since the mid-1990s a number of reports have been published in several European countries as well as in the USA and other countries, on the isolation of salmonella strains that are similar to S. typhimurium with a difference in the flagella antigen. These strains have the same O and H antigens, but lack the expression of phase 2 flagella (antigenic formula 1,4,[5],12:i:-).

They are therefore called 'monophasic S. typhimurium'. These strains have been shown to have similar virulence and antimicrobial resistance characteristics as strains of S. typhimurium.

Recent studies in numerous countries worldwide confirm the rapid emergence and dissemination of such strains in food animals, companion animals and humans.

Monophasic S. typhimurium has been included into the European Union target for the reduction of the prevalence of certain salmonella serotypes in laying hens.

This is especially important due to the multi-resistance to several antibiotics used for poultry and human therapy that is often found in these strains which are in many cases ESBL (extended spectrum beta lactamase) producers.

The efficacy of Salmonella typhimurium strain Nal 2/Rif 9/Rtt, which is the S. typhimurium component in AviPro Salmonella Vac T and AviPro Salmonella Duo in young chickens was determined by carrying out a challenge infection with a monophasic S. typhimurium field strain. Results obtained demonstrated that shedding of the monophasic S. typhimurium field strain was significantly reduced in the vaccinated chicks in comparison to the non-vaccinated control group. It could further be demonstrated that the bacterial count in the caeca and spleen was strongly reduced in the vaccinated birds.

Some European studies suggest a common link of human infections with monophasic S. typhimurium to pork and pork products; it is possible to assume that extended use of the vaccine in Europe in past years avoided higher prevalence of monophasic S. typhimurium in poultry.

Conclusions

In conclusion, a third generation salmonella vaccine is available with AviPro Salmonella Duo as the first bivalent salmonella live vaccine providing homologous protection against the main salmonella serovars present in chicken eggs and meat: S. enteritidis or S. typhimurium, as well as against the emerging monophasic S. typhimurium.

The vaccine is registered for chickens, turkeys and ducks. ■