An update on live vaccines and nutritional products in poultry

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The number of annual salmonella infections in humans is still high worldwide. A worldwide egg associated salmonellosis pandemic started in the 1970s and is currently fading away, thanks to huge efforts of policy makers and the poultry industry. This pandemic was caused by the serotype Salmonella enteritidis. Due to its preferential association with laying hen eggs, combined with the way humans tend to store (room temperature), handle and eat (non-cooked) eggs, Salmonella enteritidis had and still has a major impact on human health.

As well as enteritidis, the serotype typhimurium is also a concern. This serotype is a major meat (porcine and poultry) contaminant, and is of concern because strains from this serotype are often carrying antibiotic resistance genes.

In addition to the serotypes enteritidis and typhimurium, more than 2000 other serotypes exist, but these are less frequently associated with human food poisoning cases and outbreaks.

For example, serotypes from serogroup C, such as virchow, hadar and infantis, are often found in broilers. Some serotypes have emerged, such as Paratyphi B varietas Java in broilers in Western Europe, and the monophasic variant of Salmonella typhimurium, i.e. Salmonella 4,12:i,-.

Layers and salmonella

Although to a lesser extent other serotypes can also infect and colonise laying hens, Enteritidis is the predominant serotype found in eggs. The high prevalence of serotype enteritidis in table eggs is not completely consistent with the serotype distribution in laying hens.

The fact that different non-enteritidis serotypes can be isolated from 25-50% of the salmonella infected laying hen flocks, while more than 90% of all isolates from eggs are serotype enteritidis strains implies that the serotype enteritidis harbours some intrinsic characteristics that lead to a specific interaction with either the reproductive tract of chickens, or the egg components.

Generally, eggs can be contaminated by salmonella on the outer shell and inside the egg. The former could potentially occur due to the presence of salmonella in the hen's environment or passage of the egg through the cloaca. The latter could be a consequence of either shell penetration or colonisation of the reproductive tract of laying hens and thus incorporation in the forming egg.

Salmonella enteritidis is much more capable of colonising the reproductive tract compared to other serotypes, and is superior in surviving in the antimicrobial egg white, explaining why this specific serotype has been so successful in contaminating eggs.

As the serotype enteritidis is the main egg contaminant, this serotype is the one that needs to be controlled in layers, in addition to typhimurium, that is also sporadically found in eggs.

Broilers and salmonella

Many more different serotypes are circulating in the broiler population as compared with laying hen flocks. The heavy contamination of broilers during the live phase is also reflected in meat contamination after slaughter.

With respect to prevention of human salmonella infections, in theory all serotypes should be controlled in primary poultry production, as all of these can potentially be transmitted to humans by meat contamination in the slaughterhouse.

There is however no clear relation between the serotype distribution in broiler flocks and broiler meat, and the proportion of human salmonella infections that is caused by consumption of broiler meat (relative to egg consumption) cannot be easily estimated.

On the other hand, serotypes typically found in broiler flocks and meat, and not in other animal species (such as serotypes hadar, infantis and virchow) cause a (low) proportion of human salmonellosis cases. Other serotypes often found in broiler meat, in contrast, are not frequently causing human salmonellosis. This makes it difficult to speculate about the importance of salmonella strains and serotypes present in broiler flocks with regard to human illness.

It is clear that serotypes enteritidis and typhimurium can also be isolated in broilers and these serotypes are of concern because they are frequently associated with human food poisoning.

For the other serotypes, close monitoring of the prevalence in human cases is of utmost importance to be able to evaluate their significance.

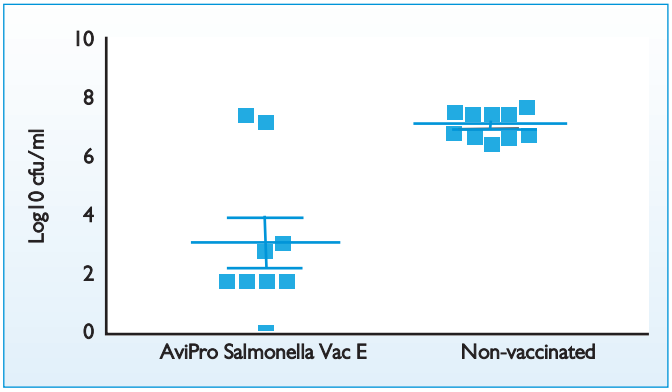

Fig. 1. Quantitative determination of the bacterial count in the caecum after oral challenge infection with 10^6 CFU of a Salmonella enteritidis field strain at two days of age.

Vaccination

Vaccination can be done using live attenuated or inactivated vaccines, and vaccines should:

- Reduce or prevent the intestinal colonisation resulting in reduced faecal shedding and thus egg shell contamination.
- Prevent systemic infection resulting in a decreased colonisation of the reproductive tissues, in this way reducing internal egg contamination.

It is very well documented that both killed and live vaccines can reduce shedding of salmonella in poultry. Although it is very difficult to prove reduction of egg contamination following vaccination under field conditions owing to the variable percentage of contaminated eggs laid, in different countries a serious reduction in salmonella prevalence in laying hen flocks and in human salmonellosis cases was observed following implementation of a vaccination programmes in layers.

Live vaccines, such as AviPro Salmonella Vac E, AviPro Salmonella Vac T and AviPro Salmonella Duo, were already proven to decrease oviduct colonisation and egg contamination. Live vaccines are believed to confer better protection because these also stimulate cell-mediated immunity. An ideal salmonella vaccine (strain) should possess the following characteristics:

- A high degree of protection against systemic and intestinal infection.
- Against a variety of important serovars (serogroups).
- Adequate attenuation for poultry, other animal species, humans and limited excretion in the environment, as well as animal welfare issues.
- The inactivated and live vaccines should not affect growth of the animal.
- Vaccine strains should not be resistant to antibiotics.

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- Vaccines should be easy to administer and need to have markers facilitating the differentiation from salmonella wild-type strains.
- Application of vaccines should not interfere with salmonella detection methods.
- Humoral antibody response after vaccination should be distinguishable from a salmonella wild-type response to allow the use of serological detection methods.

It is thus clear that salmonella vaccines are very useful in breeders and laying hen flocks and can contribute to a decrease in colonisation, shedding, and egg contamination, when the above mentioned vaccine characteristics are fulfilled.

In addition, oral administration of salmonella wild type and attenuated strains can confer resistance to infection by a virulent salmonella challenge strain within 24 hours of administration. This 'competitive exclusion'-like phenomenon is called colonisation-inhibition (CI).

It is thus possible to administer live salmonella vaccine strains to newly hatched chicks such that they would colonise the gut extensively and very rapidly, inducing a profound resistance to colonisation by other salmonella strains of epidemiological significance, which may be present in the poultry house or may have arisen from the hatchery.

Long lasting protection

Colonisation of the gut by the colonisation-inhibition strains would prevent gut colonisation by virulent strains, while invasion in the gut tissue would evoke an inflammatory response that would prevent invasion to the internal organs by virulent strains.

It has been shown that this protection can last up to slaughter age in broilers. This colonisation-inhibition mechanism can thus protect broilers, breeders and layers starting from the early post-hatch period.

Thus, these two characteristics can also be included in the list of vaccine criteria:

- Attenuated live salmonella vaccine strains should be able to induce a rapid colonisation inhibition effect.
- Attenuated salmonella vaccine strains should have preserved the ability to invade the gut.

In one study the vaccine strain within AviPro Salmonella Vac E when applied from the first day of age (10^9 CFU) has demonstrated this inhibitory effect after oral challenge infection with 10^6 CFU of a Salmonella enteritidis field strain at two days of age. The caecal colonisation level at day seven had at least 1,000 fold reduction with AviPro Salmonella Vac E in comparison with the control group.

In a different study, the efficacy of three live salmonella vaccines (AviPro Salmonella Vac E, AviPro Salmonella Vac T, AviPro Salmonella Duo) was demonstrated when reducing internal organ colonisation, including reproductive tissues after intravenous challenge infection with a Salmonella enteritidis field strain.

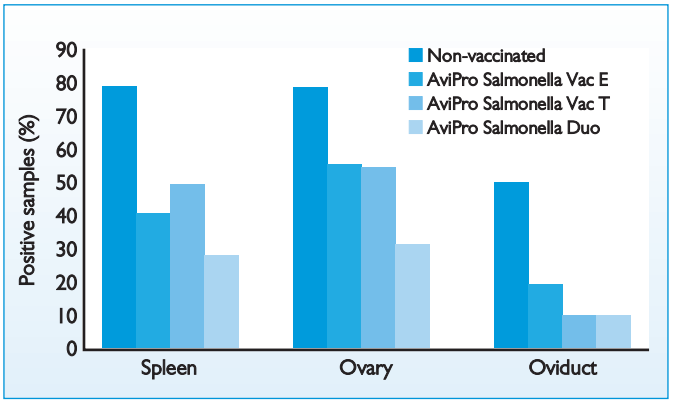

Fig. 2. Quantitative determination of the bacterial count in internal organs after intravenous challenge infection with 10^6 CFU of a Salmonella enteritidis field strain.

Other control methods

While vaccination is the most important tool to reduce salmonella colonisation in poultry, it is not 100% protective and it needs to be combined with other methods to further limit salmonella colonisation.

Especially for broilers, where classical vaccination is not commonly implemented, other methods are crucial. First of all, good farming and hygienic practices need to be conducted, in order to avoid introduction of salmonella on the farm or reduce the infection pressure when salmonella is present.

Hygienic measures at all levels of the production chain are essential for successful salmonella control: pre-harvest (during life), harvest (catching and transport) and post-harvest (processing plant). Hygienic measures should take into account feed, birds, drinking water, environment, management, cleaning and disinfection.

Vaccination of the parent broiler flocks can be used to decrease the susceptibility of the offspring by stimulating an immune response through maternal antibodies. More important however will be the application of control products in the laying hen or broiler flocks.

Mainly feed additives are of importance in this regard, since they can have effects on intestinal colonisation by salmonella.

These are mainly of importance in broiler flocks, as these are commonly the most feasible measures that can be applied in these animals during the live phase, as vaccination is not always possible due to the wide variety of serovars involved and the reduced time to build up a protective immune response (although the colonisation-inhibition principle can in theory be used).

There are an impressive number of commercially available compounds that can be used as feed or drinking water additives to control salmonella. Some have well documented effects, some less so. Most have been selected by trial and error, and the observed effects are on an empirical basis.

Feed additives used for salmonella control either have antibacterial effects or induce gut microbiota shifts that limit salmonella colonisation. Most profound antibacterial effects of course are induced by classical antibiotics, but this is not recommended as antibiotics tend to induce a carrier state in chickens and the use of antibiotics to control salmonella is not allowed in certain countries (EU for example).

Acidifying compounds also have antibacterial effects, depending on the acids used. Short-chain fatty acids, such as formic, acetic, propionic and butyric acid, have limited antibacterial effects, but medium-chain fatty acids (capric, caproic, caprylic, lauric acid) are more antibacterial. They can be used to limit salmonella entry in the host, by sanitising the drinking water and affecting bacterial survival in the crop when used as a drinking water additive.

Botanicals and essential oils are also now dominating the feed additive market. These products, such as oregano and rosemary extracts, are antibacterial as they contain aldehydes, phenols, alcohols, terpenes, and many more chemical compounds. Typical examples are thymol, carvacrol and cinnamaldehyde.

Many feed additives have an effect on the gut microbiota composition, typically steering the microbiota composition to one that suppresses salmonella colonisation.

Generally, that means that these products stimulate competition for substrates and stimulate the production of anti-salmonella signals.

A typical example of the latter one is butyrate, a signal that reduces the ability of salmonella to invade intestinal epithelial cells. Invasion in epithelial cells is a key feature that is responsible for gut colonisation and thus decreasing invasion will reduce colonisation.

While butyrate in coated or micro-encapsulated form in the feed is able to decrease salmonella colonisation, pre- and probiotic compounds can also be of value because they can stimulate butyrate formation by the already present microbiota in the gut.

Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the hindgut. Well known prebiotic products added to poultry feed are manno-oligosaccharides (MOS), glucans, xylo- and arabinoxylo-oligosaccharides (XOS and AXOS). Also fructo-oligosaccharides (FOS) and inulin could be of value.

Many preparations are available on the market, and for some products experimental proof and validation of effects on salmonella colonisation of the broiler gut is shown.

To be able to evaluate the efficacy of these products, one needs to request data from the producers. Probiotics by definition are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance.

Most well-known products are based on lactic acid producing bacteria, such as Lactobacillus species, and more recently a variety of Bacillus species have been brought to the market. Lactic acid can be consumed by a variety of strictly anaerobic bacteria to produce butyrate; the latter could explain the effects of lactic acid bacteria. Also in the case of probiotic substances, producers should show statistically interpreted efficacy data.

Competitive exclusion products, i.e. freeze-dried preparations of the whole gut microbiota, are not often used but are very effective in salmonella control. In addition, as mentioned above, protected acids can be used to bring acidic compounds in the hindgut, thereby limiting salmonella colonisation.

Conclusion

To conclude, it is advisable to use a combination of methods, vaccination, nutritional strategies,and biosecurity measures, in order to maximise the chance of reducing salmonella colonisation. Only this holistic approach will enable the poultry industry to reduce the prevalence to near-to-zero levels. ■

References are available from the author on request