

# Live salmonella vaccines and the role of secretory IgA and cell immunity

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Salmonella remains a major problem for the broiler and egg sectors of the poultry industry worldwide both in terms of disease, resulting in morbidity and associated reduced productivity but also mortality, and also from the point of view of salmonella as a zoonotic infection causing food-poisoning, which itself can have an effect on the economics of the industry.

Control of salmonella infections is affected by geo-climatic factors. Thus, although in the northern hemisphere much of the intensive rearing is done within enclosed housing and with increasing high quality feed, housing and management, there is an increasing interest in some countries towards extensive, free-range and organic farming with resulting reduced opportunities for controlling the environment.

In countries where the ambient temperature is high there may also be limited opportunities for environmental control and this may be combined with poorer management and feed quality.

It is clear that in conditions where exposure to sources of salmonella is frequent, biological measures including vaccination will necessarily form a major part of a holistic approach to infection control.

## Salmonella biology

The salmonella types that cause animal and public health disease problems are all different serotypes (also called serovars) within the species *Salmonella enterica*. Serotypes are differentiated by the type of the cell wall antigen, also called the O antigen and the flagella antigen, also called the H antigen.

There are many types of both antigens, so, for example, *S. enteritidis* contains the 9 O and g,m H antigens. *S. typhimurium* contains the 4 O and 1,2 and 1 H antigens, thereby differentiating it from enteritidis.

The poultry specific type *S. gallinarum* and *S. pullorum* are unusual in that although they

share the 9 O antigen with *S. enteritidis*, they are completely non-motile and therefore do not express flagella antigens.

From the point of view of infection biology the more than 200 serotypes may be divided into two main groups. One small group of serotypes are able to produce severe typhoid-like disease in adult animals.

They colonise the gut poorly and are therefore rarely isolated from the food chain. They include the avian-specific serotypes *S. gallinarum* and *S. pullorum*, *S. typhi* in man, *S. dublin* in cattle etc.

The other group comprising the large majority of remaining serotypes rarely cause typhoid-like disease and do colonise the gut well, thereby entering the human food chain causing food-poisoning.

These include *S. enteritidis*, *S. typhimurium*, *S. infantis*, *S. heidelberg* and many more.

## Epidemiology of infection

The epidemiology of salmonella food poisoning has been dominated during the last 25 years by the pandemic of *S. enteritidis* associated with broilers and layers.

Control instituted in the late 1990s included the use of vaccination. Combined measures have resulted in a huge reduction in infection both in birds and man on the European continent with some variation between member states.

The current situation involves predominance, albeit at lower isolation rates, of *S. enteritidis* and *S. typhimurium* with isolation of other serotypes such as *S. infantis* in some Eastern European countries and the appearance of a monophasic *S. typhimurium* in which the flagella antigen is present in only one phase and may be indicative of ongoing microbial evolution.

## Infection biology

Experimental oral infection of chickens or mice with food-poisoning serotypes, such as *S. typhimurium* and *infantis* show typical pictures of infection in both host species. *S. typhimurium* is excreted in the faeces at a

high rate but after 2-3 weeks is eliminated until at 6-8 weeks it has virtually disappeared.

*S. infantis* is excreted for much longer periods and is not eliminated for 15-20 weeks. *S. infantis* is less invasive for chickens after oral infection and stimulates lower titres of circulating specific IgG.

This suggests that the difference between the two serotypes relates to the stronger immune response induced by *S. typhimurium* which eliminates it more quickly.

The ability to clear salmonella quickly from the intestine increases with age reaching a maximum at about six weeks of age. The main site of colonisation in the alimentary tract of the chicken are the caeca and ileum. Salmonella colonise the lumen but also interact closely with the mucosa.

In fact, most bacterial growth and multiplication takes place close to the mucosa where nutrient and oxygen concentrations are at their greatest but where the corollary is that the salmonella bacteria must also endure the antibacterial products of the mucosa including secretory IgA and defensins peptides.

For entry into the caeca the bacteria must pass through the caecal tonsil which is a cluster of lymphoid tissue at the ileo-caecal junction.

We believe that this organ controls entry and exit of contents and bacteria and is able to sample the microbial contents which begins the immune response induced in the lymphoid follicles of this organ.

The very tight control means that the bacteria have very close contact with the mucosa and it is here that immune control can be at its strongest with additional involvement of cell mediated immunity.

## Immune clearance

After clearance from the intestine, chickens are relatively immune to reinfection. Similar levels of protection can be induced by live, attenuated strains of the same serotype but not generally by killed bacteria, even if these are present in the intestine in numbers similar to those present in a chicken infected

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with a live strain. Some live attenuated mutants of salmonella are also able to protect against systemic infection.

Thus, an attenuated *S. enteritidis* could protect against egg infection and an attenuated *S. gallinarum* strain (same serotype) was able to do the same. *S. gallinarum* belongs to the typhoid group of salmonella strains and it has been known for a long time that protection can be induced against these by using attenuated strains.

Protection amongst these strains is more straightforward since their epidemiology is simpler, not involving other animals species and gut colonisation is not involved in disease.

There are also fewer serotypes to protect against, in contrast to the >2000 food-poisoning serotypes, thus making protection against these more problematic.

## Vaccination and the EU

The EU under the advice from EFSA recommend the use of vaccination as part of a comprehensive approach to salmonella control. Live vaccines can be used safely provided that detection methods are able to differentiate the vaccine strain from other wild-type strains.

They can be used safely throughout the life of the bird except during the withdrawal

period before slaughter and during lay. Their use should be mandatory in those countries where high prevalence of infection is current.

Live vaccines are more effective than killed vaccines because:

- They stimulate both cell-mediated and humoral (antibody-based) immunity.
- They persist longer in the tissues thereby providing a strong immune stimulation.
- They are easier to administer through water, spray or feed.
- They stimulate innate immunity and provide some protection via this means from 24 after administration.
- They colonise the gut providing an exclusion mechanism.
- They provide at least some cross protection between serotypes, although the extent of this is not clear and most of it garnered from mouse experiments.
- Depending on the vaccine they may not interfere with serological monitoring.

## Immunity to salmonella

Soon after infection salmonella (and other bacteria) interact with Toll-like and other receptors on the host mucosal cell surface which informs the host that infection is taking place. This precipitates a cascade of pro-inflammatory cytokines (including IL-1, IL-6 and IL-8 homologues) which induce a cellular inflammatory response. This tends to limit salmonella to the intestine to some extent.

The O and H antigens are important in this regard and a non-flagellate mutant of a salmonella is able to avoid this inflammation and is more invasive. It is probably significant from that point of view that *S. gallinarum* is not flagellate and invades without inflammation.

We have found that both antibody and cell-mediated immunity is important in controlling infection in chickens. Similar studies were found much earlier with salmonella infection in mice with systemic infection. Interestingly we have also found this to be true for intestinal infection.

Following infection IgM appears first in the serum followed by IgG and IgA. IgA appears in highest concentrations in the gut although IgG and IgM are also present this is largely as a result of seepage from the blood. In the bile which empties into the gut only significant concentrations of IgA are present.

After re-infection with the same strain strong protection occurs which correlates with higher antibody titres. Protection also correlates with high IFN and TGF levels in the spleen and caecal tonsil. Smaller amounts are present on re-challenge indicating the importance of antibody in addition to cell-mediated immunity.

In contrast, MIP secretion in the intestine, which attracts macrophages, is higher in protected birds indicating the importance of cellular killing. It is clear that both cell-mediated immunity and secretory antibody

are vital to protection in the intestine and both are stimulated by wild strains and live, attenuated vaccine strains.

## The carrier state

Some serotypes such as *S. pullorum* and, to some extent, *S. enteritidis*, are able to show persistent infection after infection of young birds, despite high levels of circulating specific IgG. We are not completely sure what is happening but it may be that early infection, perhaps arising from vertical transmission is associated with induction of tolerance to infection.

Following experimental infection at a few days of age, *S. pullorum* persists within splenic macrophages in a small number of birds until at sexual maturity they begin to multiply in this organ in females (but not males) and spread to the reproductive tract resulting in infected eggs.

This event is associated with reduced T-cell (T-lymphocytes are essential to cell-mediated immunity important in controlling systemic infections such as pullorum disease) responsiveness as a result of high levels of circulating sex hormones.

We are not sure what the immune mechanism is but have shown that whereas, as indicated earlier, immune clearance is normally associated with high IFN levels we find much lower levels in *S. pullorum* infection and intermediate levels in *S. enteritidis* infection. In contrast, high levels of IL-4 are found (this is associated with high antibody levels but poorer cell-mediated immunity).

## The ideal vaccine

The ideal vaccine should:

- Provide strong protection against intestinal and systemic infection.
- Show stable avirulence for poultry with no effect on growth rate or other production parameters.
- Show stable avirulence for poultry and man.
- Generate long lasting protection (maximum generally thought to be 6-9 months).
- Protection against more than one serotype.
- Be easy to administer.
- Be eliminated from the gut after induction of immunity and thereby not enter to food chain, enable differentiation from field strains of salmonella.
- Should be compatible with other control measures.

## Additional benefits

Live vaccines, administered orally, multiply well in the intestine of young birds and as a result of this generate an exclusion effect against several salmonella types, providing a

very rapid protection mechanism until normal adaptive immunity is generated after two weeks or so.

The protection is not only against intestinal colonisation but against any mortality that might normally be induced by other virulent salmonella strains.

## Conclusion

- Immunity to salmonella infection in chickens is a major feature of normal infection biology clearing the major serotypes, *S. enteritidis* and *S. typhimurium* from the intestine and tissues, including reproductive tract.

- Live vaccines generate stronger protection against intestinal and systemic salmonella infection than killed vaccines
- Cross protection against different serotypes is limited requiring vaccination against each of the major problem serotypes to obtain full protection.
- Live vaccines generate non-specific protective effects by stimulating innate immunity and intestinal exclusion.
- Vaccines must be used as a component of a holistic and comprehensive set of control measures. ■

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*References are available from the author on request*

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