Immunity to *Salmonella* Infections

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**Introduction**

Salmonellosis is one of the most prevalent foodborne zoonoses worldwide and food animals are reservoirs for non-typhoid (Zoonotic) *Salmonella* infections. In poultry, host-specific *Salmonella* infections cause fowl typhoid and pullorum disease that produce great economic losses in different parts of the world. Several measures are involved in the prevention & control of *Salmonella* among which vaccination is the most practical measure because it avoids the contamination of poultry products and prevents infections in humans. *Salmonella* vaccines can decrease public health risk by reducing colonization and organ invasion (including invasion of reproductive tissues) and by diminishing fecal shedding and environmental contamination.

**Primary and secondary lymphoid organs**

The immune system is a highly complex physiological system and this manuscript is aiming to describe the major components of the avian immune system and their immune response. Generally speaking, whether or not a bird develops a disease after an invasion will depend on the following factors:

1. The bird’s condition, state of wellbeing and level of immunity
2. The number of the invading organisms involved in the challenge
3. The virulence or strength of the invading organisms.

In the case of birds, these immune mechanisms are developed in specific organs:

- Primary lymphoid organs: thymus, bursa, bone marrow
- Secondary lymphoid organs: gland of Harder, spleen, tonsillar tissue (Ileo-cecal tonsils), lymph nodes and the mucosa-associated lymphoid tissue (MALT) which is the diffusion system of small concentrations of lymphoid tissue found in various sites of the body, such as the gastrointestinal tract, thyroid, breast, lung, salivary glands, eye, and skin. The components of MALT are subdivided into the following specialised tissues:
  - GALT (gut-associated lymphoid tissue. Peyer’s patches)
  - BALT (bronchial-associated lymphoid tissue)
  - NALT (nasal-associated lymphoid tissue)
  - CALT (conjunctival-associated lymphoid tissue).

**Immunity in two steps**

The immune system is able to confer immunity by two steps: the 1*st* step is the innate (unspecific) immune response and the 2*nd* step is the adaptive (specific) immune response.

The 1*st* innate and unspecific response is based upon several conditioning factors such as: the genetic predisposition and the defense mechanisms (physical, microbiological, chemical and cellular defense) which are based upon:

- Genetic predisposition: some birds species may lack the receptors required by certain infecting serovars.
- Physical defense: skin, mucous membranes, cilia, bile salts, other secretions and peristalsis (coughing, vomiting, diarrhea).
- Microbiological defense: dense and stable gut microflora population prevents invading disease organisms. Improper use of antibiotics or poor sanitation can disrupt the balance of the microflora.
- Chemical defense: Low pH, cell destroying enzymes, complement system (cell communication).
- Cellular defense: intraepithelial Phagocytes (macrophages, dendritic cells, granulocytes) and leucocytes involved in inflammatory response.

The 2nd adaptive and specific response consists of humoral immunity (based upon the action of the B-cells) and cell-mediated immunity (based upon the action of cytotoxic T-cells, also known as T-lymphocytes and other leucocytes such as CD4; CD8, NK cell-digesting). The key of the immune response: the innate immune response and recognition of the agent are crucial to activate the adaptive immune response, through induction of antigen presentation and the activation of all the co-stimulatory molecules.

**Homologous vs. heterologous protection**

Generally speaking, a homologous vaccine strain shows better results when compared to a heterologous vaccine strain. The efficacy of both vaccines has been determined in several experiments by carrying out a challenge infection with *Salmonella* Typhimurium field strains at various time points during the laying period and comparing the results with those from non-vaccinated laying hens of the same age. In case of an infection with *Salmonella* Typhimurium the immunisation with a homologous vaccine strain leads to a considerable reduction in the colonisation in the caecum, the main area of localization; the reduction of the germ count in the caecum after challenge with *Salmonella* Typhimurium is remarkably higher in hens vaccinated with homologous vaccine strain than in the laying hens vaccinated with a heterologous vaccine strain.

The different *Salmonella* serovars are grouped and typed according to the Kauffmann White Scheme:

- The somatic (O antigen) determines the group
- The flagellar (H-antigen) determines the serovar within the group

Homologous protection provided by the same *Salmonella* antigenic formula is better than heterologous protection provided by a different *Salmonella* antigenic formula. Although some cross protection exists with different serovars, serovar specific protection (homologous) is superior. As a rule of thumb:

- Homologous protection is optimal: i.e. S.Enteritidis vaccine against S.Enteritidis exposure; S.Typhimurium vaccine against S.Typhimurium exposure.
- Cross protection between serovars of the same group is acceptable: i.e. S.Enteritidis vaccine against S.Gallinarum exposure (both group D).
- Cross protection between serovars of different group (heterologous) is lower: i.e. S.Typhimurium vaccine against S.enteritidis exposure.
Colonization Inhibition vs. Competitive Exclusion

Live vaccines, if administered orally, demonstrate non-specific and rapid protection against infection that is of biological and practical interest. Protective effects induced by vaccination of birds include the reduced intestinal colonisation and the diminished systemic invasion by *Salmonella* wild-type organisms. It has been shown that oral administration of live *Salmonella* strains to day-old chicks provides protection against infection with related *Salmonella* organisms within hours by an intestinal colonisation— inhibition (CI) effect which is most certainly the result of microbial physiological processes.

The term competitive exclusion (CE) is used to describe the process by which beneficial bacteria exclude bad bacteria or pathogens. CE implies the prevention of entry and establishment of a bacterial population into the gut. Oral live attenuated vaccines have a competitive exclusion effect in the intestinal tract that prevents or minimises the colonisation by *Salmonella* field strains. It should be applied to day-old chicks (not previously contaminated) in spray in the hatchery or in drinking water at farm. CE is effective in reducing *Salmonella* colonisation provided it is accompanied by conventional hygienic measures.

Immune response after field infection

Immune response against *Salmonella* depends on the host and serotype involved. Generally speaking, an intracellular bacterium is able to generate animal carriers. Reducing the challenge in the environment through complementary measures, aids in controlling systemic infections and enteritis in the long run, thus contributing less excretion, environmental extinction and food safety. The pathogenesis of *Salmonella* infection starts with the uptake of the bacteria, following the intestine colonization (ileum, ceca), then the invasion of the mucosa is produced.

- Within the gut, *Salmonella* attaches itself to the cells of the epithelium. It is known that the fimbriae and the "*Salmonella* pathogenicity islands" SPI-1 and SPI-2 play essential roles.
- *Salmonella* enters the organism either via M-cells of the Peyer’s plaques, by invading the enterocytes or by Para-cellular transportation. It has been found that the enterocytes could behave also as antigen presenting cells.
- Studies showed that *Salmonella* Enteritidis can be detected in the Lamina Propria as early as 12 hours after infection.

Once the mucosa has been invaded there are two possible routes: the first route consists of the phagocytosis of the bacteria and the consequent systemic dissemination which leads to invasion of the organs.

![Distribution of Salmonella Enteritidis and Salmonella Typhimurium in the lamina propria](image_url)

Source: Dr. Berndt and Dr. Methner (2007). Both researchers were able to show that SE and ST both distribute within the lamina proper after invading the mucosa.
The possible second route after the invasion of the mucosa is an acute inflammation (PG’s. enterotoxins, cytokines), the consequent activation of AMPc and finally the excretion of bacteria in the form of diarrhea.

- The presence of Salmonella within the Lamina Propria attracts Macrophages. They take up the bacteria via endocytosis.
- In a further step, those cells that belong to the antigen presenting cells (i.e. dendritic cells) take up the bacteria via endocytosis.
- After endocytosis, an intracellular lysis of various bacterial components occurs. As a consequence processed antigens (short peptide sequences), are presented on the outer side of the plasma membrane of the dendritic cells. This is done with the help of "major histocompatibility complexes" (MHC).
- The presentation of processed antigens on the dendritic cells induces the activation of specific T-helper cells.
- Activated T-helper cells secrete the cytokines Interleukin 2 (IL-2) and Interferon gamma (IFN-γ). The autocrin secretion of IL-2 activates an intracellular signal cascade that induces a clonal duplication of T-helper cells. The secretion of Interferon gamma activates the macrophages which start an intracellular lysis of the bacteria previously phagocytized.
- Infective Salmonella that had reached deeper layers of the tissue during earlier infection are saved from the activities of macrophages and can multiply and further invade the organism.
- Those infective Salmonella able to survive within the macrophages after endocytosis, multiply within the macrophages (Salmonella Pathogenicity Island 2 is essential for this intracellular survival). Surviving Salmonella can be transported by wandering macrophages to any place within the body of the chicken and to reach deeper layers of the tissue after being released from the macrophages. This way, infected birds can become life-long carriers that transiently shed Salmonella.

In the case of low infective challenges, once the mucosal barrier capability is weakened (selective IgA deficiency), a second line of defense would be activated. This consists of the participation and recruitment of large numbers of immune-competent cells, resulting in the onset of an inflammatory process that eradicates the antigen and functionally restores the mucosa. If this process is constant and intense, it may result in a chronic inflammatory process.

**Immune response after vaccination with MDM live attenuated vaccines**

Live Salmonella vaccines develop the cellular immunity and the local immune response (SIgA) against Salmonella infections in correspondence to the main challenges of the poultry industry: Economics, Animal welfare, Sustainability and Food quality & safety. Lohmann live Salmonella vaccines are not injected into the birds, but applied via drinking water. The application is as easy as safe, and parallels the natural path of infection. It is this combination that makes Lohmann live Salmonella vaccines so successful. Following oral uptake the vaccine enters the body through the intestinal wall for a short period. After reaching the mucosa, there is a colonization inhibition effect on the gut by
the vaccine strain which is a very favourable effect that works for about 10-12 days after vaccination. This effect is similar to the competitor excluders such as probiotics.

In the mucosa, the vaccine strains attach themselves through their flagella to the epithelial cells of the gut and try to invade the Lamina Propria just like a field strain. Within the Lamina Propria, the vaccine strains are ingested by Macrophages. These vaccine strains are so called “Metabolic drift mutants” (Metabolic drift mutants - MDM). These mutants are disabled in important metabolic pathways (due to the chromosomal attenuation) which do not allow the multiplication of the vaccine strain within the Macrophages.

In contrast to the SG9R strain (without flagella), the MDM vaccine strain through its flagellar antigens is recognized by the innate immune system (i.e. Toll-like receptor 5 - TLR5) more quickly; this is a differential feature which allows the prompt activation of the subsequent adaptive immune response. Moreover it is important to emphasize that the lack of these proteinaceous flagella in the SG9R strain demands in turn a higher frequency of vaccinations in order to stimulate properly the immune response.

In several studies it was observed that within less than 24 hours after vaccination, macrophages started to invade the Lamina Propria. Dendritic cells utilize the vaccine strains to process antigens which they present via MHC complexes on their surface to other cells. This leads to an activation of homologue T-helper cells that replicate as described for the field infection. By secreting IFN gamma (IFN-γ) the T-helper cells activate the macrophages. Then, activated macrophages start to lysate the ingested vaccine strain and present those processed antigens via MHC complexes on their surface. This induces the cytokines Interleukin 2 (IL-2) mediated production of cytotoxic T-lymphocytes which are memory specific. These cells represent the immunological memory; in case of a field infection they start a rapid multiplication and induce a strong immune response. In several studies it was observed an increase in T-cell activity in the bursa, ceca and spleen only few days after vaccination.

The secretion of Interleukin 4 (IL-4) and Interleukin 5 (IL-5) by T-helper cells induces the differentiation of B cells to plasma cells. After differentiation the new plasma cells start the secretion and release of antibodies at the Peyer patches and then into the gut lumen: thus preventing adherence of bacteria and viruses to epithelium and preventing penetration of these into sub-epithelial layers of the intestine:
- One type of antibodies is immunoglobulin M (IgM). This pentameric structured type of antibodies participates in the activation of the complement system. IgM attaches itself to the surface of invaded cells and thus enables their phagocytosis.
- Immunoglobulin G (IgG) or IgY in poultry, is also secreted by activated plasma cells.
- IgY also binds invaded cells. This either leads to a direct cytolysis of those cells or it marks them for macrophages that will ingest those marked cells.
- The main weapon against *Salmonella* is IgA, which is secreted by plasma cells and then turned into a dimer in the mucosa. This is also the time when the secretory component is attached to the dimeric IgA. This secretory component enables the transportation of the immunoglobulin through the cells of the epithelium into the gut lumen.

- SIgA (secretory IgA) represents a strong neutralizer of invading *Salmonella* in the gut lumen. The attachment of SIgA to the bacterial cells inhibits them to invade the Lamina Propria. This triggers off an immediate response in the form of local immunity. Once secretory IgA (the most efficient defense against *Salmonella* infections) is formed and released into the gut lumen, any virulent *Salmonella* pathogens in the intestine are immediately rendered harmless by the secretory IgA before they can enter the lamina propria and colonize the internal organs.

- To summarize the results, vaccination with AviPro® *Salmonella* Vac E, AviPro® *Salmonella* Vac T and AviPro® *Salmonella* Duo stimulates a strong reaction of the immune system. This includes:
  - The invasion of macrophages into the Lamina Propria.
  - A significant activation of T-lymphocytes and
  - The synthesis of secretory IgA.

Secretory IgA is the most important player in the immunological defense against *Salmonella*. Only SIgA has the ability to prevent the invasion of *Salmonella* at an early age. Therefore, vaccination with live vaccines is essential to protect birds and prevent colonization since very beginning of the life cycle of the birds.

**Immune response after vaccination with inactivated vaccines**

After a vaccination with an inactivated vaccine by subcutaneous or intramuscular injection, there is a primary humoral immune response of Ig M (7 to 14 days p.v.) followed by production of Ig G. There is a gradual rise in antibody production taking days to weeks (Ig G). Once the plateau is reached, antibody level declines. Without continue antigenic challenge, antibody levels drop off. It is a must a second exposure to the same antigen to get a booster effect. The immunological memory cells will produce high and homogeneous long lasting Ig G serological titers that will be transmitted to egg and progeny. The second administration of an inactivated vaccine, at least 4 weeks p.v., results in immediate production of protective antibody, mainly Ig G, but may be some Ig M. Ig M is the first immunoglobulin to be produced and Ig G is the most abundant
antibody found intravascularly and extravascularly and it is stored at egg yolk and yolk sac and provides humoral immunity to chick for first weeks of life.

References