Avian Salmonellosis, vaccines and immune mechanisms of protection: present and future perspectives

Liliana Revolledo
DVM, MSc., PhD.
Prof. Ad. Postgraduate Unit
Faculty of Veterinary Medicine,
University of San Marcos, Lima - Perú

Summary

Official control for animal diseases is defined by thSalmonellosis is one of the most prevalent foodborne diseases worldwide. Food animals have been identified as reservoirs for non-typhoid Salmonella infections. Several measures have been used to prevent and control Salmonella infections in poultry and vaccination is the most practical measure and effective method to control and prevent Salmonellosis. Salmonella vaccines can decrease public health risk by reducing colonization and organ invasion, including reproductive tissues, and by diminishing fecal shedding and environmental contamination. This presentation discusses present and future approaches based on scientific information regarding killed and live attenuated Salmonella vaccines and their immune mechanisms of protection.

Introduction

There is continuing interest in finding ways of preventing flock infection and, hence, contamination of poultry products with Salmonella enterica. Control measures are difficult to use effectively because there are numerous potential sources of Salmonella infection and product contamination in an integrated poultry enterprise [Revolledo et al., 2006]. Control of Salmonella infections in poultry farms needs to begin with good farming practices and appropriate management associated with strict sanitary measures. Preventive and curative strategies have been widely applied for reducing the incidence of Salmonella colonization in chickens at the farm level [Vandeplas et al., 2010]. Various prophylactic measures have been employed to prevent and control Salmonella infection in poultry production, and vaccination is one of them. Salmonella vaccination aims to mimic the development of naturally acquired immunity by inoculation of non-pathogenic but still immunogenic components of the pathogen, reducing or eliminating the risk for the consumer. Killed and live attenuated products have been used for controlling Salmonella in poultry production, thus vaccination with live attenuated products has proved to be effective [Cerquetti & Gherardi, 2000]. This is a brief overview of Salmonella vaccines in chickens, immune mechanisms of protection, and the future perspectives in this area.

Pathogenesis in poultry

Most of the known Salmonella serotypes are pathogenic for humans or animals or both. Although Salmonella pathogenesis has been well characterized in the mammalian model [Barrow, 2007], there is limited information of specific mechanisms in avian species [Dunkley et al., 2009]. Poultry species can be infected by host-specific and non-host specific Salmonella serotypes.

Salmonella Gallinarum (SG) and Salmonella Pullorum (SP) cause severe disease and death of birds compared with other known Salmonella serotypes. Chadfield et al., (2003) suggested that SG invades the bursa but the process is not time dependent, and they demonstrated no selectivity for a potential port of entry for the host specific serotype. Avian systemic salmonellosis has three phases: invasion, systemic infection and the resolution of the infection [Sadeyen et al., 2004]. The third phase can have three results: the clearance of the bacteria, death of the birds due to infection and, partial clearance of the bacteria, which leads to a subclinical carrier state, as shown in Figure 1. The biology of Pullorosis is markedly different...
when compared to Fowl Typhoid, which causes high mortality. Pullorosis induces an increase of *Salmonella* in the spleen resulting in an infection of the reproductive tract [Wigley *et al*., 2005].

*Salmonella* infects poultry and other animals by the oral route. Non-host specific *Salmonella* in poultry is frequently involved in food-poisoning in humans. In chickens, it produces systemic disease in some special cases, such as the laying period, in chicks in the first two weeks of life or after viral diseases. The pathogenesis of non-host *Salmonella* serotypes in poultry is summarized in Figure 1. *Salmonellas* are not native members of the gut microbiota, but young chicks are readily colonized, and the organisms may persist in the host for some weeks or during all of the rearing period [Revolledo *et al*., 2006]. They become localized in the cecal tonsils and can occur in the upper part of the small intestine and in the gizzard and proventriculus [Fanelli *et al*., 1971]. Because most birds infected with *Salmonella* become symptomless carriers, they constitute a reservoir of the organisms, which is a potential human health hazard. Additionally, by contaminating the environment, these birds are responsible for increasing the number of infected individuals [Revolledo *et al*., 2006].

### Immune response against *Salmonella* infection

The immune response to *Salmonella* infections is very complicated and involves the interaction of many components of the immune system including the innate and the adaptive immune system [Nagarajan *et al*., 2009]. Although progress has been made in understanding immune responses against *Salmonella* infections, further research is needed to understand the complete roles of humoral and cell-mediated immunity because until now, no consistent pattern has been observed. Pathogenic bacteria have evolved mechanisms to invade the epithelial cell barrier and survive within host tissues. *Salmonella* maintains genes organized within pathogenicity islands that encode virulence factors that allow adherence, invasion and dissemination in the host [Aziz *et al*., 2007]. TLR (Toll-like receptors) are cell receptors which recognize structural motifs on pathogens and initiate signaling cascades controlling the development of innate immune response [Chaussé *et al*., 2011]. These receptors contribute to host resistance to microbial pathogens and can drive the evolution of virulence mechanisms [Arpaia *et al*., 2011] and can

Figure 1 - Pathogenesis of host-specific and non-host specific *Salmonella* in poultry.
promote adaptive immunity through control of dendritic cell maturation [Iwasaki & Metzhitov, 2004]. The consequences of Salmonella infection on the expression of the different TLR, and particularly TLR4, have been widely studied [Crhanova et al., 2011].

Salmonella Gallinarum does not induce an inflammatory response and may not be limited by the immune system, leading to severe systemic disease [Kaiser et al., 2000]. Invasion of SG results in low or no production of IL-6 suggesting that the pathogenesis and host specificity of the SG infection in the chicken may be related to some extent to the lack of an inflammatory response in the early stages of the infection in the gut [Kaiser et al., 2000].

Chickens infected with enteric Salmonella serovars show high levels of specific antibodies, a T cell response, cytokines and chemokines. Within cell populations, their function can be further discriminated by the presence of cellular determinants, such CD4+ (T helper cells) and CD8+ (T cytotoxic cells), which are associated with helper and cytotoxic functions respectively [Jeurissen et al., 2002]. The local immune response in the gut has been shown to be more effectively involved in the clearance of Salmonella Enteritidis from the gastrointestinal tract than in the systemic response [Desmidt et al., 1998]. An important role of local cell-mediated immunity in the defense of chickens against Salmonella exposure has been suggested [Berndt & Methner, 2001], describing that modifications of T-cell populations, especially CD8+TcR+γδ (T-cell receptor-bearing cells) cells in ceca, occur few days after the inoculation of one-day-old chickens with the serovar Typhimurium.

It has been suggested that intestinal S IgA (secretory IgA) responses partially contribute to the later elimination of the Salmonella Enteritidis from the gut, and the humoral systemic and local immune responses seem to be related to the cecal colonization [Berthelot-Hérault, 2003]. Cell-mediated immunity is responsible for tissue clearance, but how this mechanism could be responsible for intestinal clearance remains unclear [Zhang-Barber et al., 1999]. The role of T cell responses in the clearance of enteric Salmonellae has not been proven. However, in the absence of an essential role for B cells (bursa-derived cells) and with faster clearance of infection as a secondary challenge, the responses are likely to be important evidence for immune memory [Smith & Beal, 2008].

Recently studies of cytokines and chemokines expression in vitro have confirmed previous work showing that paratyphoid species stimulate significant mRNA expression levels of proinflammatory IL-6, inducible nitric oxide synthase (iNOS) and chemokines [Setta et al., 2009]. It was suggested that host gene expression as well as differences between chicken lines in host responses toward the Salmonella infection, are host dependent [Van Hemert et al., 2006].

Interestingly, Berndt et al., (2007) evaluated the chicken cecum immune response and showed that low quantities of enteric bacteria were inside the macrophages. These results indicated the capability of paratyphoid Salmonella serovars to enter and invade the cecal mucosa, affecting the level and character of the immune response. The expression of IL-12, IL-18, TNF-α (tumor necrosis factor alfa), and iNOS in cecum was correlated with the invasiveness of serovars in the lamina propria. In contrast, IL-2 mRNA expression, and changes in the numbers of TCR2 (T-cell receptor 2) and CD4+ cells seem to be more dependent on the infection of intestinal epithelial cells [Berndt et al., 2007].

Crhanova et al., (2011) found out that chickens respond to natural colonization of caecum by and increased expression of IL-8 and IL-17 in the first week of life. These authors showed that chickens infected with Salmonella Enteritidis before, during and after the IL-8 and IL-17 induction, responded through Th1 (T helper cell subset 1) inducing IL-8 and IL-17, while birds infected after this point responded more through Th17 (T helper cells subset 17) branch of the immune response. These results indicate that the gut microbiota and expression of some cytokines increase the resistance to S. Enteritidis infection.

**Vaccines against Salmonella and immune mechanisms of protection**

The regulation and effectiveness of the avian acquired immune response is comparable to that in mammals [Erf, 2004]. For a better understanding of these mechanisms, an extensive review of vaccines, protection and the immune dynamics of the avian digestive system are available [Barrow, 2007; Korver, 2006]. In chickens, vaccines should prevent intestinal and cecal colonization resulting in diminished fecal shedding, and should be effective against systemic infection preventing vertical transmission and egg contamination. Vaccinations with either inactivated
or live products have been applied for reducing the susceptibility of poultry to *Salmonella* infections [Gast, 2007]. Both inactivated and live vaccines are used to protect against *Salmonella* challenge, however, killed products increase humoral immunity and reduce *Salmonella* prevalence but did not significantly decrease *Salmonella* in the farm environment [Berghaus *et al*., 2011].

**Vaccines**

Vaccines based on dead *Salmonella* bacteria have been used to protect poultry and its progeny against field challenges. Inactivation is usually achieved by either heating or the use of formalin and an adjuvant. However, there are two remaining problems with these products, they fail to elicit a cell-mediated immune response, which is considered the most important for clearing the intracellular pathogen [Lax *et al*., 1995], and they do not stimulate secretory IgA (SIgA) responses at mucosal surfaces, which is the key for protection against intestinal colonization [Cerquetti & Gherardi, 2000].

Subunits vaccines, like outer membrane proteins, porins, ribosomal fractions have been tested to prevent *Salmonella* infections. The efficacy of these products is not good when administered without an adjuvant or a delivery system [Gamazo & Irache, 2007].

Live and attenuated vaccines have been used worldwide, and their efficacy has been demonstrated in challenge trials [Atterbury *et al*., 2010; Papezova *et al*., 2008]. The aim of a live attenuated vaccine should be to reduce the bacterial virulence while maintaining its immunogenicity. Live vaccines include semi-rough strains, such as 9R [Silva *et al*., 1981; Kwon & Cho, 2011]. Other attenuated vaccines include auxotrophic and metabolic drift mutants (Table 1). Mutant vaccines attenuated by biological gene-deletion techniques include aroA mutants, *crp* mutants and others as describe in Table 1, this genetically modified organisms are permitted in the USA, and other countries, except Europe.

It is important to evaluate the behavior of the attenuated bacteria strains in the environment, focusing on the attenuated strain obtained by gene deletions, because they can acquire genes from other microorganisms and recover their virulence. The SG 9R vaccine (Table 1) strain still results in systemic disease, with pathology in the liver and

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spleen, and bacteria persist for several weeks at these sites [Silva et al., 1981]. Lypopolysaccharide (LPS) defect may be one of the major mechanisms of SG 9R attenuation [Kwon & Cho, 2011] but this defect could induce partial recognition by TLR4. Possession of intact SPI-2 (Salmonella pathogenicity island 2) and spvC (Salmonella plasmid virulence), B, A and R virulence genes may be associated with residual SG 9R virulence [Kwon & Cho, 2011] Mutations should be introduced to increase safety by reducing the risk of reversion [Linde et al., 1998].

**Immune mechanisms of protection: present and future perspectives**

Despite the fact that many studies have been carried out to demonstrate the efficacy of inactivated [Woodward et al., 2007], sub-units [, live attenuated [Atterbury et al., 2010; Kwon & Cho, 2011], bacterial ghost [Peng et al., 2011], genetically derivative strains [Nandre et al., 2011; Methner et al., 2011] used as vaccines, the efficacy of these product is demonstrated by challenge trials and recovery of the challenge strain. Little and isolated information explains how do vaccines work and the immune response depending on the antigen and route of vaccination.

Vaccines should establish a long lasting immunity by manipulating the cytokine milieu to induce the appropriate effector mechanisms for each particular pathogen and by creating a large pool of long lived memory cells [Chabalgoity et al., 2007]. Vaccines used in poultry against Salmonella infections have been effective, but they were empirically designed, and are not based on detailed information about the immune responses of protection because efficacy is frequently evaluated in challenge trials.

The route of vaccination is important in influencing immune responses at the initial site of pathogen invasion where protection is more effective [Belyakov & Ahlers, 2010]. Mucosal dendritic cells play an important role in the induction and maintenance of protective immunity against pathogens like Salmonella. Dendritic cells are responsible for antigen presentation following mucosal vaccination and systemic immunization have a limited effect on the delivery of antigen to mucosal dendritic cells [Coombes & Powrie, 2008; Kelsall, 2008].

An important difference must be established in Salmonella attenuated vaccines regarding the immune response: the administration route. Parenteral vaccines stimulate a strong humoral response, while oral live attenuated vaccines generate both mucosal and systemic immunity [Bouvet et al., 2002; Mastroeni et al., 2000]. There is a fundamental difference between inactivated and attenuated live vaccines. Live attenuated Salmonella vaccines stimulate the secretion of mediators (cytokines, interleukin and interferon) inducing a T helper 1 response and cell-mediated immunity; in different animal species and in poultry this type of immune response is not induced by inactivated products. Additionally, CD8+ T cells play a crucial role in immune protection against intracellular pathogens, such as Salmonella, and these cells (CD8+T) response are exclusive after vaccination using live attenuated vaccines.

Attenuated live bacteria vaccines applied by oral route are excellent tool for mucosal immunization [Husseiny & Hensel, 2005], because mucosal surfaces are the first interface between Salmonella and the host. The first step to initiating an immune response in the gut surface by oral vaccines is based on the signals sent by receptors for pathogen-associated molecular patterns (PAMP) via pathogen recognition receptors (PRR), such as TLR. TLR in chickens are very similar to those in mammals; however, some differences in recognition patterns related to TLR5, which recognize flagellin are observed in host-specific Salmonella and non-host-specific Salmonella strains [Salazar-Gonzales & McSorley, 2005]. In response to flagellin lamina propria propri dendritic cell, the differentiation of naïve B cells into IgA plasma cells occurs via a mechanism that is independent of the gut associated lymphoid tissue (GALT) [Uematsu & Akira, 2009]. Recognition of S. Typhimurium is largely mediated by TLR2, TLR4 and TLR5 [Uematsu et al., 2006; Vasquez-Torres et al., 1995] and TLR9 is involved in the regulation of the replication of the bacterium. Lypopolysaccharide (LPS) of Salmonella is recognized by TLR4 expressed at the surface of the immune cells and in the cytoplasm of intestinal epithelial cells [Crhanova et al., 2011]. The second step in the immune response is related to the ability of the antigen to cross the epithelial barrier and to be presented to antigen-presenting cells, especially dendritic cells. Mucosal dendritic cells play a central role in the induction of protective immunity against invasive pathogens. Unique dendritic cells subsets are responsible for antigen presentation following mucosal vaccination [Belyakov & Ahlers, 2009]. In the chicken, multiple lymphoid follicles exist, and they are made up of B cells embedded in a network of follicular dendritic cells [Brisbin et al., 2008]. The chicken epithelial barrier can be crossed using three mechanisms: endocytosis in the intestinal epithelial cells, transcytosis crossing M cells (microfold cells), and directly through the intraepithelial lymphocytes.
The third step in the immune response produced by oral vaccines is the processing by the dendritic cells and the presentation to the T cells. Dendritic cells in the gut can be activated by epithelial cells, which produce cytokines based on the invasiveness of the bacteria, and directly by noninvasive bacteria [Rimoldi et al., 2004]. *Salmonella* vaccines administered orally must induce Th1 and Th2 (T helper cell subset 2) responses, stimulating cell-mediated immunity and B cell activation for producing SIgA (Figure 2), which block the attachment of the bacteria to mucosal surfaces. To achieve protection, it is important that memory cells are generated in enough numbers and persist as a functional long-lived population [Chabalgoity et al., 2007]. Some authors indicated that it would seem crucial that the vaccine strains retain capacity of invasiveness in order to stimulate sufficient immunity to be protective [Barrow, 2007; Van Immerseel et al., 2005], however, the immune response in the intestinal mucosa has revealed new possibilities because oral antigens induce effector and memory cells that express certain receptors only on lymphocytes of intestinal mucosa [Aziz et al., 2007]. Mucosal memory T cells phenotypes differ substantially depending on the regimen of immunization, a secondary responses result in preferential accumulation of memory T cells in the lamina propria following mucosal vaccination [Barnhart et al., 1991].

These cells might be exploited to develop new live attenuated vaccines inducing abroad repertoire of immune responses against intracellular pathogens [Titball, 2008].

Vaccination is an excellent tool for handling poultry *Salmonella* prevention programs in developed and developing countries. Further research is needed to evaluate immunological interactions among the host and *Salmonella*, avoiding empirical methods in developing new vaccines and investigating ways to prevent the infection. Vaccines against *Salmonella* infections in chickens and other food-producing animals require protection at both mucosal (gut) and systemic levels. There is no ideal product, but regarding the immune response, live attenuated *Salmonella* vaccines are superior to inactivated, deleted or subunit vaccines.

![Diagram](image_url)

**Figure 2** - Mucosal protection and CMI in the chicken gut conferred by oral live attenuated *Salmonella* vaccines.
Oral *Salmonella* vaccines provide an advantage over parenteral products, because oral products are able to mimic natural infection and to stimulate both the mucosal and systemic immune responses, whereas parenteral products are able to generate only systemic immune responses. Advances and new strategies in elucidating the mechanisms of action of oral vaccines for poultry will facilitate the design of multivalent and more effective products. New design and delivery strategies for eliciting mucosal and systemic immune response are needed in order to develop more efficacious multivalent vaccines against *Salmonella* for preventing infection in the poultry and contamination in poultry products.

**References**


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