

Avian Salmonellosis, vaccines

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Abstract: Salmonellosis is one of the most prevalent food borne 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. 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 *Salmonella* vaccines and their immune mechanisms of protection.

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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 produce contamination in an integrated poultry enterprise [Revollo 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 (Vandeplass 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, and vaccination with live attenuated products has proved to be more effective (Cerquetti and Gherardi, 2000).

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 pathogen city 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 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 little 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 and Methner, 2001), describing that modifications of T-cell populations, especially CD8+TcR1+(gd) (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, et al., 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 and 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 alpha), 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.

***Salmonella* serovars in vaccination and cross protection between serovars**

As *S. Typhimurium* and *S. Enteritidis* are the serovars of *Salmonella* most important for public health in Europe all existing commercially available *Salmonella* live and inactivated vaccines are intended for use against these serovars. Also the indication of a commercial live *S. Gallinarum* vaccine strain is the active immunisation of layers against *S. Enteritidis*. However, for other serovars relevant to human infections no vaccines are available for poultry production. The salmonellae primarily responsible for enteritis in humans belong to a number of serogroups, including groups B, C and D. There is little evidence for any significant cross-protection between serogroups in mice (**Hormaeche et al., 1996**), cattle (**Meyer et al., 1993**) or chickens (**Curtiss and Hassan, 1996**) although the reason for this remains unclear. Some experimental evidence exists indicating little mutual protection between groups B and D in chickens. No published information exists for group C. It seems likely that lipopolysaccharide (O-antigen) is a major component of the key immunogenic component and that protection between strains within a serovar is likely to be much greater. This assumption is supported by investigations in poultry under both experimental (**Springer et al., 2000**) and field conditions. After introduction of large scale vaccination using live *S. Typhimurium* strain in poultry breeding farms (layers and broilers) the detection rate of both *S. Typhimurium* and *S. Enteritidis* dropped considerably. Twelve months after starting vaccination *S. Typhimurium* was no longer detected, indicating the strong homologous immunisation effect. The detection of *S. Enteritidis* was reduced but this heterologous protection was much less effective than the homologous effects against *S. Typhimurium*, suggesting only a partial cross immunity effect between serogroups B and D (**Vielitz, 1993**).

Live and inactivated *Salmonella* vaccines

Both live and inactivated *Salmonella* vaccines are available for poultry and a variety of vaccine preparations has been developed and tested for their protective efficacy in poultry (**Barrow et al., 1991**; **Cooper et al., 1992**; **Vielitz et al., 1992**; **Methner, et al., 1994**; **Curtiss and Hassan, 1996**; **Hahn, 2000**;

Springer et al., 2000; Feberwee et al., 2001). Although a number of different live *Salmonella* strains have been tested for their efficacy in experimental or semi-field studies only a few are authorised and commercially available for use in poultry in Europe. The accessible live *S. Typhimurium* and *S. Enteritidis* vaccine strains are either auxotrophic double-marker mutants derived through chemical mutagenesis (**Meyer et al., 1993; Springer et al., 2000**) or developed on the basis of the principle of metabolic drift mutations (**Linde et al., 1997; Hahn, 2000**). Some of these *Salmonella* live vaccines were further characterised by molecular methods (**Schwarz and Liebisch, 1994**). Another live vaccine authorised for prophylactic use against *S. Enteritidis* is based on a rough strain of *S. Gallinarum* without further molecular characterisation (**Feberwee, et al. 2001**). Also a number of different inactivated preparations of *Salmonella* organisms have been tested for their efficacy against *Salmonella* challenge in poultry. However only one commercial inactivated *S. Enteritidis* based vaccine against *S. Enteritidis* infection in breeders and laying type chickens (**Feberwee et al., 2001**) is used in different countries and one commercial inactivated bivalent *S. Enteritidis* and *Typhimurium* dual vaccine against both *S. Enteritidis* and *Typhimurium* has been authorised (**Clifton-Hadley et al., 2002**). These killed vaccine types are based on bacterial cells cultured under conditions of iron depletion. Vaccination schemes using a combination of live and inactivated *Salmonella* vaccines have been shown to be effective. Usually live vaccines are administered orally via drinking water in very young chicks during the rearing period followed by parenteral injection of inactivated vaccines before the beginning and during the laying period. However, immunisation schemes that do not use combination of live and inactivated vaccines are also used (**Vielitz, et al., 1993; Feberwee et al., 2001**). A vaccine containing inactivated *S. Enteritidis* that was grown under iron-restricted conditions is available on the market in some European countries (**Woodward et al., 2002**). Also, a vaccine containing *S. Enteritidis* as well as *S. Typhimurium*, both grown under conditions of iron restriction, is also commercially available (**Clifton-Hadley et al., 2002**). Subunit vaccines have also been used in poultry. Outer-membrane protein vaccines with adjuvant have been used to decrease shedding of *S. Enteritidis* in poultry (**Meenakshi et al, 1999**). **Khan et al. (2003)** immunized 9-week-old chickens with two outer membrane proteins subcutaneously, followed by two boost immunizations with time intervals of 2 weeks. These outer membrane proteins were shown to be involved in attachment of *S. Enteritidis* to intestinal epithelial cell lines (**Fadl et al., 2002**). Immunization of either of the outer membrane

proteins decreased caecal colonization about 1000-fold when the animals were infected orally with 8₁₀₈ CFU virulent *S. Enteritidis* strain (**Khan et al., 2003**). Attention has been paid to the development of a virulent vaccine strains of *Salmonella* because of the accumulation of evidence that such strains of *Salmonella* are more immunogenic in mice and in poultry than are killed or subunit vaccines (**Collins, 1974; Zhang-Barber et al., 1999**). Live vaccines have been tested extensively in mice and also in poultry. Although a number of different live *Salmonella* strains have been tested for their efficacy in experimental or semi-field studies, only a few are registered and commercially available for use in poultry in Europe. The commercially available live *S. Typhimurium* and *S. Enteritidis* vaccine strains are either auxotrophic double-marker mutants derived through chemical mutagenesis (**Meyer et al., 1993; Springer et al., 2000**). The use of live attenuated *Salmonella* strains to deliver recombinant antigens to the immune system is an attractive additional strategy for the creation of multivalent vaccines for poultry. Multivalent vaccines would decrease the number of vaccinations required in the field. Sustained expression of the heterologous antigen in the tissues in an immunogenic form at levels sufficient for priming a protective immune response is the main target when developing *Salmonella* recombinant vaccines (**Mastroeni et al., 2000b**). Vaccination of chickens with a Dcyacr_p mutant of *S. Typhimurium* expressing the *Escherichia coli* O78 lipopolysaccharide O-antigens induced antibodies against the O78 lipopolysaccharide Oantigen and against *Salmonella*, and engendered a degree of protection against challenge with a pathogenic *E. coli* O78 strain (**Roland et al., 1999**). *S. Typhimurium* vaccine strains were used as antigen delivery system for oral immunization of chickens against two antigens of the coccidian parasite *Eimeriatenella* (**Pogonka et al., 2003**). However, the delivery of antigens to the immune system is not sufficient per se to engender a protective response. A successful vaccination also requires the elicitation of an appropriate type of immune response. Thus, different groups are working on the development of carrier-based vaccination strategy in order to promote this. For example, strains carrying mutations affecting the specific course of infection can be exploited to modify the immune response elicited (**Drabnerand Guzman, 2001; Dietrich et al., 2003**), or the sub-cellular location of recombinant antigen in the vaccine *Salmonella* strain may influence the type of the immune response (**Kang and Curtiss, 2003**). In addition, the co-delivery of immunestimulatory molecules facilitates triggering a predictable response according to specific needs (**Dunstan et al., 1996**). This type of work has, up to

now, been performed only in mice. For example, **Igwe et al. (2002)** constructed a chimeric protein based on the Yersinia outer protein E (YopE) comprising the listerial antigens eliciting a cell mediated immune response. In mice orally immunized with attenuated Salmonella vaccine strains expressing the chimeric Yop E trans located by the type III secretion system, this novel vaccination strategy led to the induction of a pronounced cytotoxic CD8 T-cell response that conferred some protective immunity against Yersinia (**Russmann, 2004**).

Immune mechanisms of protection:

Vaccines should establish a long lasting immunity by manipulating the cytokine milieu to induce the appropriate effect or mechanisms for each particular pathogen and by creating a large pool of long lived memory cells (**Chabalgoity et al., 2007**). The route of vaccination is important in influencing immune responses at the initial site of pathogen invasion where protection is more effective (**Belyakov and Ahlers, 2010**). Mucosaldendritic 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 and 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**) Attenuated live bacteria vaccines applied by oral route are excellent tool for mucosal immunization (**Husseiny and 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 and McSorley, 2005**).

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