Novel Recombinant Food Safety Vaccines against *Salmonella* and *Campylobacter* in Broiler Chickens

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Abstract

Salmonellosis and campylobacteriosis are the most common food borne diseases occurring worldwide. In addition to the human health significance and associated treatment costs, Salmonellosis and Campylobacteriosis can also result in economic loss from trade disruptions as well as recall of large consignments of poultry products. Among the various potential sources, poultry meat and eggs are identified as major sources for these organisms. *Salmonella* and *Campylobacter* are usually commensal organisms seen in the gastrointestinal tract of poultry, which makes control difficult. The use of vectored vaccines, targeted to both systemic and mucosal immunity systems, and containing immune enhancing molecules, is a promising approach in enhancing the food safety of poultry products. Novel recombinant vaccines were developed using vectors such as double – attenuated *Salmonella Enteritidis* and *Bacillus subtilis*. Highly conserved antigenic epitopes on the target organisms were identified and inserted into these vectors. In addition, the vectors were also engineered to express immune enhancing molecules CD154 and/or high mobility group box 1 (HMGB1) on their cell surface. Broiler chickens were vaccinated and the immune responses, including both IgG and IgA levels, and/or protection from the target organism were measured. The recovery of *Campylobacter jejuni* from poultry was reduced by the administration of *Salmonella*-vectored as well as *Bacillus* – vectored *Campylobacter* vaccines. Furthermore, both vaccines increased the levels of C. jejuni – specific IgG, while *Salmonella* – vectored vaccine also increased the C. jejuni – specific IgA levels. Meanwhile, in another study, a *Bacillus* – vectored vaccine...
elicited protection against S. Typhimurium in some treatment groups. Thus, novel food safety vaccines have the advantages of reducing colonization of these pathogens in host tissues, harmless to host, compatible with other control measures, no food residues, and easy – cost effective way of administration.

**Keywords:** Salmonella, campylobacter, food borne diseases, recombinant vaccines, poultry.

### 1. Introduction

According to WHO, around 1.8 million people died in 2005 due to consumption of contaminated food and drinking water, and associated food borne illness (Newell et al., 2010). Salmonellosis and campylobacteriosis are two major food borne diseases occurring in many regions of the world and most common sources for these organisms are identified as poultry meat and eggs. *Salmonella* and *Campylobacter* are seen in gastrointestinal and/or reproductive tract of birds as commensal organisms which could contaminate meat or egg products leading to diseases in human beings. Like any other bacteria, these organisms can also be controlled by adding antibiotics in poultry diets. However, growing concerns over the use of antibiotics in food animals due to the possibility of antibiotic residues and concerns about the development of antibiotic resistant strains of pathogenic organisms have encouraged producers to seek alternative means of control. The main objective of this manuscript is to discuss about potential use of novel vaccine strategies, based on the results from our laboratory, to improve food safety in poultry industries.

### 2. Strategies to Develop Effective Food Safety Vaccines

Vaccines are typically developed against antigenic regions or epitopes present on target organisms. When administered to host organism, the immune system of the host will recognize these epitopes, become activated and prevent or reduce the spread of target pathogenic organisms. However, developing vaccines becomes challenging when there exist multiple serovars of pathogenic organisms expressing individual epitopes that elicit little or no cross-protection against other serovars. For example, there are more than 200 serotypes of *Salmonella*, with different antigens, capable of infecting both domestic animals and human beings (Hargis et al., 2010; EFSA 2010). Firstly, an effective food safety vaccine should make use of highly conserved and universal epitopes on target organism resulting in broad spectrum protection against various serovars.

Secondly, food borne illness causing organisms like *Salmonella* and *Campylobacter* are non-invasive and colonize poultry guts without causing any apparent disease conditions. So, conventional vaccines administered parenterally or as injection have little significance in controlling these organisms, as these vaccines will
stimulate mainly humoral (IgG and IgM) and cell-mediated immune responses in host with little mucosal immunity. In order to reduce the colonization of *Salmonella* and *Campylobacter* in gastrointestinal tracts, mucosal immunity (secretory IgA) in hosts needs to be stimulated, which will help to prevent the infection and colonization of these microorganisms.

In order to overcome these constrains, recombinant vaccines using attenuated live vectors are used which can be administered orally to stimulate mucosal immunity. These vectors are genetically modified to express universal epitopes for the target organism so that broad spectrum protection is obtained. The attenuated bacteria vectors used for recombinant vaccines are not able to infect or cause disease in host organisms. As a result, the immune response elicited by these vectored vaccines may not be robust enough. However, this disadvantage of weaker immune response can be overcome by the co-expression of immune enhancing molecules on the vectors along the universal epitopes. Thus, the immune cells on the mucosal surface of gastrointestinal tract can respond to the immune enhancing molecules along with the target antigen leading to an enhanced immune response. Several studies reported the immune regulatory effect of CD154 (CD40L), a tumor necrosis factor ligand expressed on activated T-cells (Barr et al., 2003; Xu and Song, 2004; Tregaskes et al., 2005). Up regulation of various immune-stimulatory molecules as a result of the interaction between CD40 and CD154 will activate antigen presenting cells and T-cell mediated immune response (Grewal and Flavell, 1998; Miga et al., 2000; Xu and Song, 2004). Another potential immune enhancing molecule which can be used in vectored vaccines is high mobility group box1 (HMGB1) protein which is reported to initiate and enhance both innate and adaptive immune responses (Ulloa and Messmer, 2006). A number of studies have been conducted in our laboratory, so far, testing various immune enhancing molecules in recombinant vaccines (Layton et al., 2009; O’Meara et al., 2010; Shivamaraiah et al., 2010).

### 3. Use of Vectored Vaccine Platform for Food Safety

Recently, molecular biological techniques are used to develop recombinant vaccines using genetically modified bacterial vectors expressing heterologous target antigen (Ashby et al., 2005; Ceragioli et al., 2009; Deguchi et al., 2009). One of the major bacterial vectors used for developing recombinant vaccines is attenuated *Salmonella*, due to the advantages of low cost of production and ease of administration through different routes such as drinking water or spray application. *Salmonella*, when administered orally, can colonize ceca and liver/spleen by 12h and 24-48h, respectively (Cheeseman et al., 2008), thus stimulating mucosal, humoral and cell-mediated host immunity (Ashby et al., 2005). Studies conducted in our laboratory tested the use of attenuated recombinant *Salmonella enterica* serovar Enteritidis PT13A isolate for developing vectored *Campylobacter* vaccines expressing antigenic candidates CjO113 (Omp18/CjaD), CjO982c (CjaA), and CjO42O (ACE393) (Layton et al., 2011). The live *Salmonella*-vectored *Campylobacter* was administered orally in
day old chicks, and 21d later followed by *Campylobacter jejuni* challenge. Among the various vaccine candidates tested, CjO113-vaccinated chicks had no *C. jejuni* recovered from ileum samples, and had higher slgA and serum IgG antibodies when compared to other groups (Layton et al., 2011). This observation was in accordance with the study conducted by Godlewska et al. (2009) reporting an increased humoral response with CjO113 vaccination obtained from a *Salmonella* plasmid vector.

Similarly, *Bacillus subtilis* (BS) is another vector used in our laboratory for developing recombinant vaccines against both *Salmonella* and *Campylobacter*. Studies were conducted by vaccinating chickens with *Bacillus subtilis* (PY79)-vectored CjO113 vaccine co-expressing CD154 in comparison to saline control and *B. subtilis* backbone. Two doses of vaccine (10^6 or 10^8) were tested with a primary vaccination on 1d of age followed by two boosts at 10d and 21d of age. The birds were challenged with *C. jejuni* (1.5 X 10^8/bird) at 24d and ileal recovery was tested at different time points (Figure 1). Birds vaccinated with the BS-vectored CjO113 showed lower ileal recovery of *C. jejuni* (Figure 1A) and higher serum IgG (Figure 1B) when compared to saline control and BS backbone. In another study, a live BS vectored vaccine expressing NNP (19 aminoacid synthetic peptide sequence) and HMGB1 was tested against saline control, BS backbone and NNP-conjugated with keyhole limpet hemocyanin (KLH, Gen-Script Corp.) administered along with chitosan adjuvant to evaluate protection against *Salmonella* Typhimurium. Birds were vaccinated on 1d (primary vaccination) and boosted on 10d followed by *S. Typhimurium* challenge (10^8cfu/chick) on 14d of age. Blood samples were collected from these birds on 17d and 21d of age. The study showed that administration of both BS vectored vaccine and conjugated-NNP resulted in lower recovery of *S. Typhimurium* when compared to saline control and BS backbone (Figure 2).

**Figure 1**: Ileal recovery of *Campylobacter jejuni* (A) and CjO113-specific serum IgG (Sample/positive ratio) levels (B) from chickens vaccinated with saline control, *Bacillus subtilis* backbone (BSBB), *Bacillus* (Py79)-vectored CjO113 co-expressing CD154 vaccines (10^6 or 10^8).
**Figure 2**: Recovery of S. Typhimurium from birds vaccinated with saline control, *B. subtilis* backbone (BS Py79), live *B. subtilis* NNP co-expressing HMGB1, and NNP-conjugated with KLH administered with chitosan.

### 4. Conclusion

Two major concerns regarding developing food safety vaccines against *Salmonella* and *Campylobacter* in poultry are the prevalence of a large number of serovars of these organisms, which limits the spectrum of protection from any conventional vaccine strategies, and colonization in gastrointestinal tract of poultry without causing any disease, thus mucosal immunity has to be given prime importance. Studies from our laboratory showed that *Salmonella*- or *Bacillus*- vectored vaccines expressing highly conserved antigenic regions of food safety target organisms could enhance both the mucosal (sIgA) and humoral (serum IgG) immunity, resulting in reduced colonization of these pathogenic organisms. In addition, vectored vaccines with co-expression of immune stimulatory molecules like CD154 and/or HMGB1 may be a valuable technique to fortify the immune response.

### References


