Vaccination against *Salmonella enterica* Enteritidis using Siderophore Receptor and Porin Proteins

*Cox, G.J.M., Griffith, B., Reed, M., Sandstrom, J.D., Peterson, M.P. and Straub, D.E.

Epitopix, LLC. 1801 Biotech Ave NE, Willmar, MN 56288
*Corresponding author: Graham.Cox@Epitopix.com

Summary

Salmonella remains a major food safety concern with poultry products. Whole-cell bacterin vaccines are widely used to protect commercial poultry flocks, but their effectiveness is limited by two factors. First, whole-cell bacterin vaccines include a wide range of proteins that are not immunoprotective. Second, each vaccine only protects against the specific serotypes included in its formulation. While autogenous vaccines can extend to cover serotypes not commonly found in commercial products, they are still limited to their source serotypes. In contrast, Siderophore receptor and porin-based (SRP®) vaccines are directed towards a much smaller set of proteins to focus the immune response against iron acquisition; a significant pathogenic mechanism of bacteria. Further, SRP's are highly homologous proteins and hold the promise of broad protection unlike those based on serotype or somatic antigens.

Willmar Poultry Company has a 20-year history of successful vaccination against Salmonella in breeder turkeys using a siderophore receptor-porin (SRP) vaccine and recently, Epitopix, a subsidiary company, has developed a new *Salmonella enterica* Enteritidis (SE) vaccine for use in chickens using the same technology. Vaccinated and placebo control chickens were challenged with SE and subsequent shedding and reproductive organ colonization were examined. Shedding was significantly reduced in vaccinates compared to controls and there was complete protection of the reproductive organs compared to the placebo group. This represents the first step towards an inactivated vaccine expected to be broadly protective for Salmonella species in chickens.

Background

Iron is an essential cellular nutrient required for electron transport, thus energy production, and for enzymatic cofactors involved in essential metabolic processes. As such, iron acquisition is essential for gram-negative bacterial growth; a prerequisite for colonization and pathogenesis. However, iron is severely limited during microbial invasion of a host species because the host forms iron complexes with high affinity iron-binding proteins such as transferrin in blood or lactoferrin in secretory fluids that make iron generally unavailable. This lack of availability of iron within a host is a barrier to infection that microorganisms must overcome to proliferate. To counter this, bacterial pathogens have evolved specific molecules for obtaining iron from their hosts. Bacteria secrete small iron-chelating molecules, called siderophores, that bind iron with high affinity and "steal" iron directly from the host binding proteins. Bacterial porin proteins on the outer membrane are receptor proteins for iron-siderophore complexes and facilitate the internalization of iron. The siderophore receptors and other porins, collectively referred to as the SRPs, are surface exposed, highly conserved and expressed in high copy number on the outer membrane. These traits make SRP good antigens for vaccine development because they provide targets for antibody that can block iron uptake and facilitate complement lysis and phagocytosis.

Materials and Methods

General protocol: This vaccine efficacy study was a placebo-controlled, randomized and double blind study to demonstrate the effectiveness of an SE bacterial extract vaccine to protect chickens from SE colonization. Hens were vaccinated subcutaneously with 0.25 mL of vaccine in the back of the neck at 10 weeks of age and again 8 weeks later. Control hens were similarly injected with a placebo vaccine containing adjuvant but without antigen. Hens were challenged 4 weeks later. Hens were swabbed in the cloaca twice weekly until Day 13 of the challenge period, after which they were necropsied and their reproductive organs harvested. All samples were tested for the presence of the challenge organism by plating on brilliant-green nalidixic acid (NAL) agar. Data were analyzed by Preventive Fraction and Mitigated Fraction statistical methods (1).

Chickens. 100 Specific Pathogen Free leghorn hens were obtained from Valo BioMedia, (Adel, IA) and grown to 10 weeks of age. The hens were tagged with numbered wing bands, randomly allocated into groups and commingled for the duration of the study.

Challenge. The challenge organism was a recent US field isolate and its identity was confirmed by the Minnesota Poultry Testing Laboratory. The challenge organism was propagated in broth cultures and all hens were challenged with $\geq$ 1 x 10^8 colony-forming units (CFU).

Results

Shedding of the challenge organism was monitored from cloacal swabs over 13 days after challenge. All hens were negative prior to challenge. On all days, the number of hens shedding was significantly reduced in vaccinates compared to placebo treated hens (Preventive Fraction analyses: Day 2 p=0.037, Day 6 p=0.002, Day 9 p=0.007 and Day 13 p=0.016). In addition, the severity of colonization of placebo treated hens was compared to vaccinates by Mitigated Fraction analyses. Consistent with the incidence data, the CFU values for the vaccinated group were significantly different than the placebo group on all four sample days after challenge.

On Day 14 after challenge, all hens were necropsied and their reproductive organs were harvested and tested for the presence of SE. The placebo group had a reproductive organ colonization incidence of 24%, which was consistent with previously reported challenge studies with SE (2,3) (Figure 1). No ovaries or oviducts from the vaccinated group were colonized with SE (Preventive Fraction analyses: 100% protection, p=0.016).

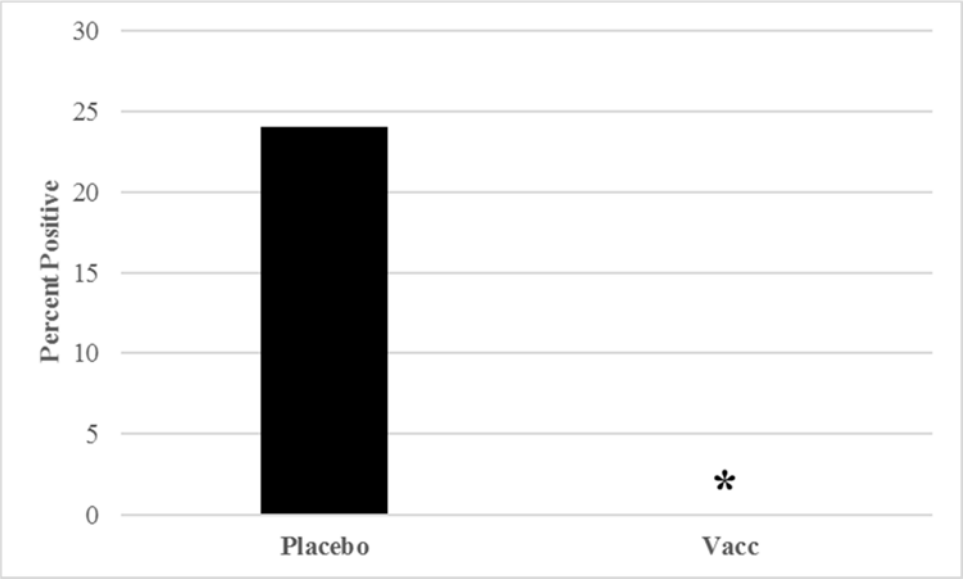

Figure 1. Prevention of SE Colonization of the Reproductive Organs by Vaccination. Percentage of hens positive for colonization of the reproductive organs with SE 14 days after challenge administration. * Indicates significant difference of vaccinates from the placebo group.

Discussion

SE continues to be a problem in the poultry industry and the incidence is expected to increase as the use of antibiotics declines and as the movement to cage-free aviaries increases. Therefore, as part of effective control programs, the use of vaccines will intensify and it is important to provide the industry with current technologies that will be effective in this changing environment. An SRP® vaccine is such a technology, eliciting immunity directly focused on a main pathogenic mechanism of SE; that is, its ability to acquire iron, and eliminating much of the irrelevant immunity and reactivity elicited by bacterins.

The SRP technology has been used in several commercial poultry operations for over 20 years in autogenous form to successfully control Salmonella, *E. coli* and fowl cholera. It is also used in the licensed bovine Salmonella Newport (4) and E. coli (5) vaccines where a reduction of shedding, reduced disease and improved herd performance have been reported. Similarly, Epitopix has gained USDA approval of efficacy for a Klebsiella mastitis vaccine (6) shown to reduce infection and to improve herd performance as well as a cross-protective *Pasteurella multocida* vaccine for chickens (reported in these proceedings).

In this study, the SE SRP vaccine was demonstrated to reduce cloacal shedding and prevent colonization of the reproductive organs. Reducing shedding is critical to prevent spread of Salmonella by the fecal-oral route in hens and the subsequent contamination of eggs and poultry for human consumption. Specific prevention of ovary and oviduct colonization is critical to prevent vertical transmission and contamination of eggs for consumption. Therefore, the ability of the SRP vaccine to effectively reduce shedding and prevent reproductive organ colonization confirms the utility of this vaccine in Salmonella control programs.

References

1. Siev, D. An estimator of intervention effect on disease severity. J Modern Applied Statistical Methods, 4:500-508. 2005.
2. Gast, Richard K. and C.W. Beard. Isolation of Salmonella enteritidis from Internal Organs of Experimentally Infected Hens. Avian Diseases 34:991-993. 1990.
3. Gast, Richard K., Stone, Henry D., Holt, Peter S. and C.W. Beard. Evaluation of the Efficacy of an Oil-Emulsion Bacterin for Protecting Chickens against Salmonella Enteritidis. Avian Diseases 36: 992-999. 1992.
4. Hermesch, D.R. *et al.* Effects of commercially available vaccine against Salmonella enterica serotype Newport on milk production, somatic cell count, and shedding of Salmonella organisms with no clinical signs of salmonellosis. Am J Vet Res. 69: 1229-34. 2008.
5. Thomson, D.U., *et al.* Use of a siderophore receptor and porin proteins-based vaccine to control the burden of Escherichia coli O157 in feedlot cattle. Foodborne Pathog Dis. 6: 871-7. 2009.
6. Gorden, P.J., Kleinhenz, M.K., Ydstie, J.A., Slinden, L. and Burkhardt, D. Evaluation of a Novel Vaccine Based on Siderophore Receptor Proteins and Porins (SRP ® Technology) for Controlling Klebsiella Mastitis in a Dairy Herd. American Association of Bovine Practitioners conference proceedings (submitted for publication). 2016.