Vaccination and Serological Response of (Siderophore Receptor and Porin Proteins) SRP® Under Natural Field Conditions

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INTRODUCTION

Escherichia coli and *Salmonella* enterica are gram-negative bacilli belonging to the Family Enterobacteriaceae. *E. coli* is considered normal flora of the poultry intestinal tract with specific strains designated as Avian Pathogenic *E. coli* (APEC). APEC is the primary agent causing Colibacillosis, a common poultry disease in commercial layer and broiler breeder hens contributing to high morbidity and mortality. Common clinical signs are airsacculitis, pericarditis, cellulitis, and peritonitis. The economic impact on the poultry industry is significant worldwide. Salmonellosis is a disease in poultry caused by multiple serotypes of *Salmonella,* ranging from mild clinical symptoms to chronic illness. Birds surviving a challenge to the organism may remain in a carrier state, transmitting the bacteria horizontally and vertically. According to the United States Department of Agriculture, the disease is a significant health concern in the human food chain as a leading contributor to human foodborne illness. Antibiotic resistance is becoming increasingly prevalent, making vaccination the most relevant source for preventing and controlling pathogenic *E. coli* and *Salmonella*. SRP technology is a novel vaccine based on purified siderophore receptors and porin proteins (SRP®). When used as antigens in the formulation of vaccines, these cell surface proteins have been shown to promote immunity to prevent Colibacillosis and Salmonellosis in poultry. This vaccine evaluation will be the basis of this study presented.

SRP® technology is a unique process utilizing specialized transport proteins called siderophore receptors, a class of porin proteins located on the outer surface membrane of bacteria. Siderophore receptors transport iron across the bacteria membrane providing essential nutrients to the cell. Bacteria are grown in an iron-restricted environment to enhance the expression of siderophore receptors and porin proteins. These proteins are extracted from the whole-cell bacteria to form a purified protein vaccine.

MATERIALS AND METHODS

This study looked at the serological response of broiler breeder laying hens to vaccination. Five flocks were included in the study, each consisting of approximately 12,000 hens. At 17 to 18 weeks, the pullets received a 0.25mL intramuscular injection. The vaccine composition comprised siderophore receptor and porin proteins isolated from *E. coli* and *Salmonella*, formulated in an oil adjuvant. To evaluate the serological response to vaccination under natural field conditions, sera were collected at 11, 20, 29, 39, and 50 weeks of lay, post-vaccination. The immunological response to vaccination was evaluated with an ELISA using purified siderophore receptor and porin proteins as the capture molecule in the assay. The antibody titers were measured using an S/P ratio, as illustrated in Figures 1 and 2.

Two *E. coli* isolates were selected based on their SRP profiles using SDS PAGE Gel electrophoresis, which determined the number and variation of SRPs used to formulate the vaccine composition, as shown in Figure 3. We have shown that SRP expression is highly variable between different serotypes of *E. coli*. Due to the conserved nature of SRPs amongst multiple serotypes of *E. coli*, antibodies generated from the SRPs of one isolate can cross-react immunologically to other *E. coli* serotypes, as shown in the western blot in Figure 4.

RESULTS AND DISCUSSION

The serological response to vaccination was monitored by ELISA. Each serum sample was run individually using the SRPs derived from the two *E. coli* isolates as the capture molecule. The results are shown in Figures 1 and 2. Please note the duration of the antibody response to the SRP antigens through 50 weeks of monitoring. This increased duration in the antibody response is due to the conserved nature of these proteins. We have shown that once the bird is vaccinated and primed immunologically to these proteins, any subsequent subclinical infection with *E. coli* expressing SRPs on their outer membrane will activate an anamnestic response, increasing the duration of immunity. All flocks were monitored daily during the study for mortality and bi-weekly for environmental *Salmonella*. Four flocks-maintained baseline mortality, while one of the five experienced a spike at 27 weeks of age. The root cause was determined by necropsy as infectious peritonitis. By 29 weeks, the mortality had returned to the baseline level. During the routine surveillance, no *Salmonella* was detected in any of these five flocks.

The variation in molecular protein patterns between *E. coli* serotypes is shown by gel electrophoresis, Figure 3. These patterns are used to choose the best antigens to represent the variability across a group of *E. coli* isolates. The Western Blot shown in Figure 4 shows that when used in a vaccine, the two SRP-derived *E. coli* antigens induced B-cell production of antibodies that bind to multiple other *E. coli* SRP surface proteins.

CONCLUSION

Siderophore receptor proteins are highly conserved across gram-negative bacteria, indicating cross-reactivity among the variable *E. coli* isolates. SRP® antigens used in vaccination show the ability to reduce the prevalence of *E. coli* and *Salmonella*. There was an 80% reduction in mortality due to the protection of colibacillosis in hens vaccinated with SRP-derived antigens, Figure 5.

Figure 1:

ELISA assay evaluating serological response in hens vaccinated with SRP *E. coli* antigens.

Sera were collected at 11, 20, 29, 39, and 50 weeks of age. Titers represented by Sample-to-Positive (S/P) ratio.

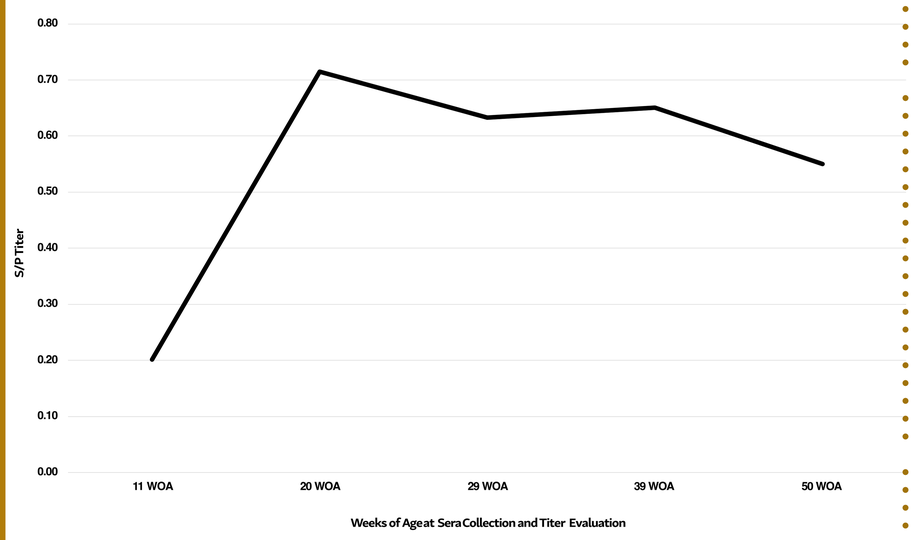


Figure 2:

ELISA assay evaluating serological response in hens vaccinated with SRP *Salmonella* antigen.

Sera were collected at 11, 20, 29, 39, and 50 weeks of age. Titers represented by Sample-to-Positive (S/P) ratio.

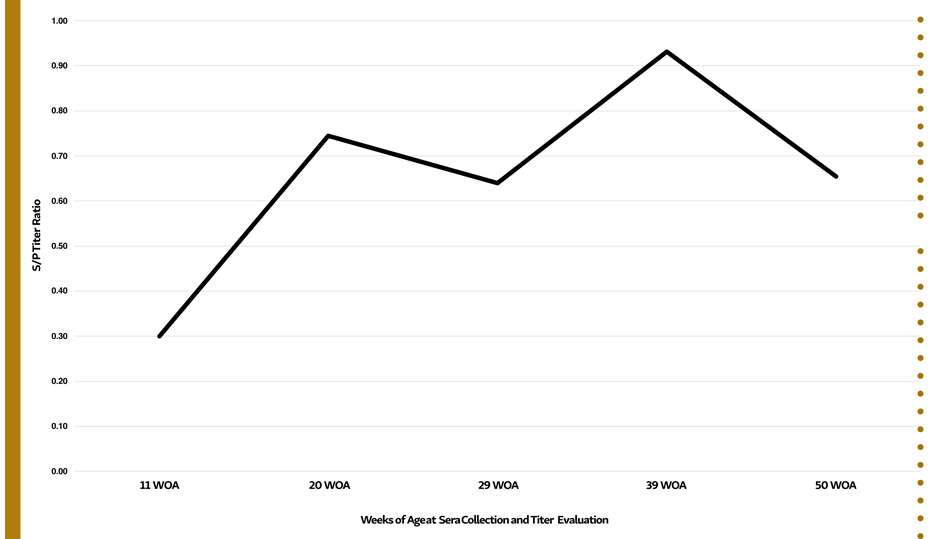


Figure 3:

Common *E. coli* (APEC) isolates gathered from Avian species. Siderophore receptor and porin proteins are separated by molecular weight in each APEC isolate using SDS PAGE Gel Electrophoresis.

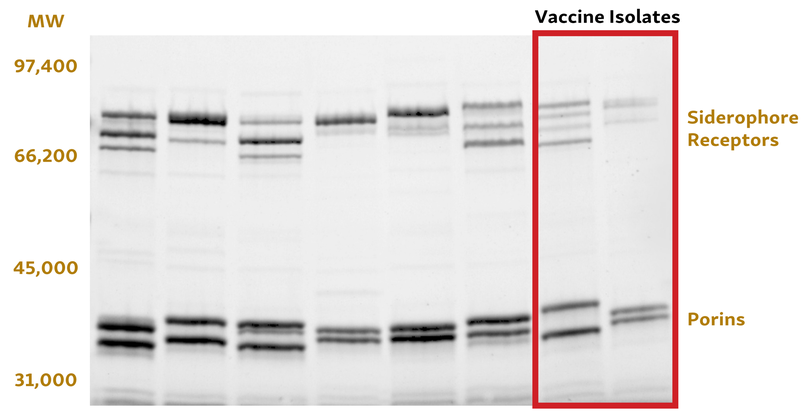


Figure 4:

Western Blot showing primary antibodies induced by the vaccine isolates binding to *E. coli* (APEC) antigens from the SDS PAGE Gel Electrophoresis in Figure 3.

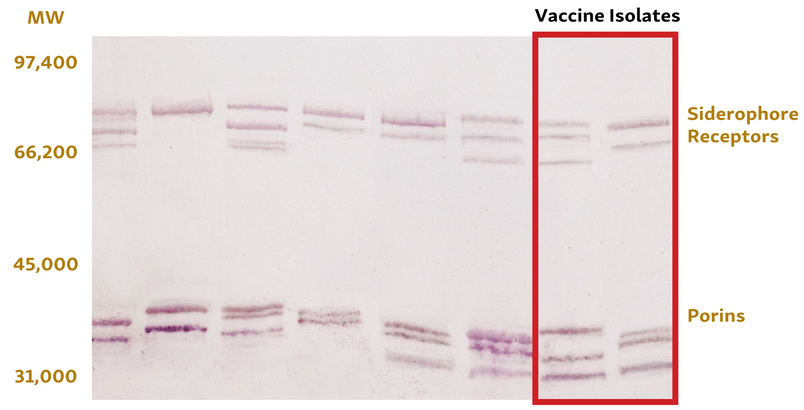
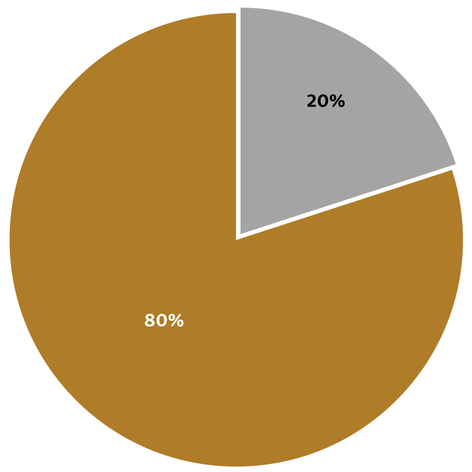
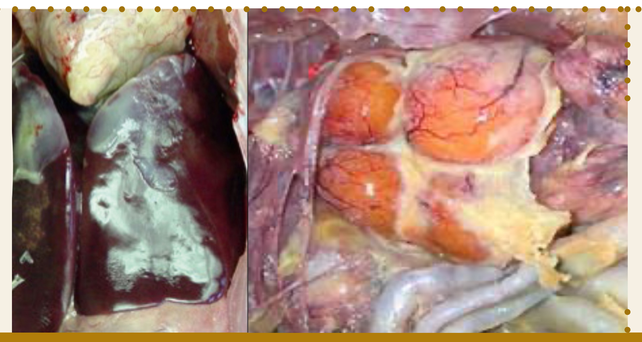


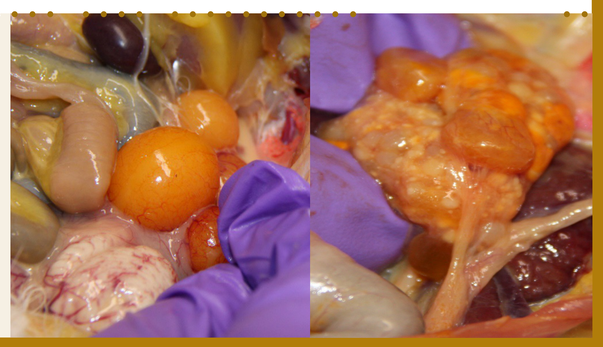
Figure 5
80% Protection with SRP® = 80% Increased Livability



Colibacillosis:
The image depicts a liver (left) an ovary (right) of poultry infected with APEC.



***Salmonella*: Healthy versus Infected**
Healthy ovary (left)
Salmonella-infected ovary (right)



References upon request