Vaccination with Siderophore Receptors and Porins Protects against Fowl Cholera Challenge by Heterologous Serotypes

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Summary

Fowl cholera is a contagious lethal disease affecting both wild and domestic fowl caused by *Pasteurella multocida*. Conventional vaccines are widely used to protect commercial poultry flocks. For typical commercial bacterins to be effective, whole cells of one to four common serotypes are included. These bacterins typically only protect against serotypes included in the vaccine. Autogenous whole-cell bacterins may also be used to protect against serotypes not commonly found in commercial vaccines.

Epitopix has developed a new fowl cholera vaccine using the company's Siderophore Receptor and Porin (SRP®) technology. The vaccine was produced using a serotype 3x4, and a serotype 2x5 strain of *Pasteurella multocida,* which when combined were identified to contain the appropriate SRP composition. Birds were vaccinated with Pasteurella SRP, divided into two groups of vaccinates and placebo control chickens and challenged IM with either *Pasteurella multocida*, a serotype (7, 9, 10) strain, or a serotype (8, 14, 15) strain. Results of these Challenges gave 92% and 70% prevention of mortality, respectively, in vaccinates compared with control birds. This study conclusively shows cross serotype protection using a SRP technology based Pasteurella multocida vaccine.

Background

Historically, commercial inactivated Pasteurella multocida vaccines have based their protective immune response on O-polysaccharide associated with each strain's serotype. Unfortunately, vaccines based on this strategy are serotype specific and thus only confer protection against the serotypes included in the vaccine. Rather than basing our vaccine on O-polysaccharide, Epitopix SRP Pasteurella vaccine's protective response is based on iron acquisition outer membrane proteins and porins.

The ability to survive and proliferate successfully within its host is a necessary characteristic of any bacterial pathogen. The host defends against bacterial invasion by creating a hostile environment for the bacterium, which includes conditions such as limited oxygen availability, osmotic and pH stress, the presence of antimicrobial enzymes, and nutrient restriction. Many of these obstacles are overcome by the protective role of the major porin proteins located in the outer membrane of gram negative bacteria that allow solute diffusion or mediate active transport. The four major groups of porins are the general porins; the monomeric porins; the specific porins; and the TonB-dependent, gated porins (iron-siderophore/hemophore receptor proteins). The various porin types allow pathogens to grow in diverse environments. For example, iron is a primary nutrient required by most bacteria for electron transport and for cofactors for essential metabolic enzymes. Iron is severely limited during microbial invasion of a host species; the host forms iron complexes with high affinity, iron-binding proteins such as transferrin in blood, lactoferrin in secretory fluids, ovotransferrin in albumin, and ferritin within cells. The low availability of iron within a host is a barrier to infection that microorganisms must overcome in order to proliferate. Bacterial pathogens have developed strategies for obtaining iron from their hosts. A common method bacteria employ to acquire iron is the production of receptors in the outer membrane which bind the host iron-bound molecules and interact with other membrane-associated proteins to internalize the iron. These 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 SRPs good candidate antigens for cross protective vaccine development.

Materials and Methods

General protocol: The vaccination/challenge protocol was a modified procedure adapted from 9CFR 113.117 potency test for Pasteurella Multocida Bacterin, Avian Isolate, Type 1(1) (Animal and Plant Health Inspection Service, US Department of Agriculture).

Chickens. Specific Pathogen Free leghorn chickens were obtained from Valo BioMedia, (Adel, IA) and grown to 12 weeks of age. The chickens were divided into four groups of 21 with 10 females and 11 males in each group. Individual birds were identified by numbered wing bands. Band numbers were blocked by sex and randomized. All chickens were housed together and provided antibiotic-free feed and water ad libitum.

Vaccination. Chickens were vaccinated subcutaneously in the back of the neck at 12 weeks of age and again 21 days later. Groups received vaccine and challenge as listed in table 1. Vaccinate groups received SRP vaccine prepared from a 3x4 serotype and a 2x5 serotype of *Pasteurella multocida*. Placebo groups received vaccine containing adjuvant and saline, but no vaccine antigens.

Table 1: Treatment and Challenge Groups

Group	Vaccine treatment	Challenge Serotype
A	SRP serotypes 2x5 and 3x4	7, 9, 10
B	Placebo	7, 9, 10
C	SRP serotypes 2x5 and 3x4	8, 14, 15
D	Placebo	8, 14, 15

Challenge. The challenge organisms were recent field isolates causing disease in commercial poultry operations. The strains were confirmed Pasteurella multocida by PCR and identified to serotype by agar gel immunodiffusion test. Cultures were grown overnight at 37°C in trypticase soy broth and adjusted to an appropriate level of colony forming units (CFU) for challenge. Challenge counts were confirmed by serially diluting the culture and plating each dilution on TSA II 5% sheep blood agar plates, in duplicate. Twenty birds of each group were challenged in the breast muscle with 0.5 mL 14 days following the second vaccination. Extra birds were removed from the study at this time.

Observations: Chickens were observed for mortality daily for 7 days. Any moribund birds were euthanized per the Center for Veterinary Biologics Testing Protocol SAM 607 (2) (USDA Center for Veterinary Biologics).

Results

At a challenge dose of 5.0 x10^8 CFU, both field strains were highly virulent (50% mortality for serotype 7,9,10 and 65% mortality for serotype 8,14,15). Protected fraction was 70% against serotype 7x9x10, and 92% against serotype 8x14x15(table 2). The two-sided p-value, as calculated by Fisher's Exact Test, was 0.04 for the 7x9x10 challenge, and 0.0001 for the 8x14x15 serotype challenge, indicating a significant and extremely significant difference, respectively.

Table 2: Mortality results of challenge

7,9,10 Challenge	# Dead/ # Tested	% Mortality	Prevented Fraction
Vaccinate	3/20	15%	70%
Placebo	10/20	50%	
8,14,15 Challenge			
Vaccinates	1/20	5%	92%
Placebos	13/20	65%	

Discussion

Fowl cholera is common across the globe and is known to be transmitted by common birds and animals. Infected flocks can very quickly realize high mortality, as well as a significant decrease in productivity in layers and breeders. While treatment and removal of infected birds is required for control, strict biosecurity and vaccination programs are central to the prevention of large outbreaks of disease in commercial flocks.

There are generally two types of commercial vaccines for fowl cholera: killed bacterins and live attenuated vaccines. Attenuated live vaccines have short lived immunity so the need for multiple revaccinations is common. Attenuated live vaccines may also cause illness in some birds post-vaccination. Previous research has been unable to demonstrate serotype cross protection against *P. multocida* by bacterins produced in iron-depleted medium (3) (Glisson, et. al.). Producers often turn to autogenous killed vaccines in response to disease and death in flocks vaccinated with commercially available fowl cholera vaccines which, while they offer better protection for serotypes specific to the farm site, do not protect against serotypes that are not present in the vaccine.

The SRP technology has been used in several commercial poultry operations for over a decade in autogenous form to successfully control fowl cholera, and the technology has been validated to work under field conditions using the licensed bovine *Salmonella* Newport vaccine (4) (Hermesch, et. al.), and conditionally licensed bovine *Escherichia coli* Bacterial Extract vaccine (5) (Thomson, et. al.) produced by Epitopix.

The present study provides an important demonstration that vaccine made with the SRP proteins of *Pasteurella multocida* provides broad protection against field strains of heterologous serotypes of virulent *Pasteurella multocida*. This vaccine has met efficacy requirements of the USDA Center for Veterinary Biologics against serotype 1 in chickens, and is currently in safety trials across the USA. This technology continues to show promise to protect against multiple serotypes of *Pasteurella multocida* in commercial poultry operations.

References

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