**INTRODUCTION**

Routine annual or semi-annual vaccination in horses is a common standard of care and is required by the governing bodies of equine sports and other aspects of the equine industry. Since the West Nile Virus (WNV) vaccine was introduced into practice, Type I hypersensitivity reactions including fatal anaphylactic shock have been on the rise.

These Type I hypersensitivity reactions are caused by an induced response of IgE to the non-target antigens within the vaccine, and not viral components of the vaccine. The IgE binds to the mast cells then to the antigen when it is injected. The IgE then cross-links, communicating to the mast cells to release histamine and other allergic mediators into the tissue. The release of these mediators can cause adverse effects to both respiratory and gastrointestinal systems. Anaphylactic shock is caused by a contraction of the bronchial smooth muscles, causing closing of the airway. An induced IgE response can also cause the contractions of smooth muscles in the gut, producing symptoms of colic.

The non-target antigens responsible for inducing these IgE responses in horses are found in the remnants of the medium that is used to grow the virus for killed and attenuated vaccines. In the case of most equine vaccines, including the killed virus for WNV vaccine, the industry standard medium for growing virus is in Fetal Bovine Serum (FBS). FBS contains many serum proteins, the most abundant protein being Bovine Serum Albumin (BSA). In 2012 it was shown via ELISA that BSA was reactive components of fetal calf serum in dogs that developed allergic reactions after non-rabies vaccination. Veterinary Immunology and Immunopathology. 2012; 151: 48-54.

During this experiment, it was determined that a 3 uL vol of FBS as well as an SDS cut to remove IgG from sera to be reacted with anti-IgE optimized the Western blots. Both IgE and IgG were used as secondary antibodies. Bands were not seen with IgG but were seen with IgE. At a dilution of 1:7000 using the commercial HRP conjugated horse anti-IgE antibody, no bands were seen. The next step would be to further concentrate the horse anti-IgE antibody at a dilution of 1:500. Another possibility would be to use the same unconjugated horse anti-IgE antibody that was used in the ELISAs that was made in the Gershwin lab. Using this indirect antibody could possibly enhance our ability to see bands.

**RESULTS**

Sera from horses in previous experiments that showed an increased induction of IgE to BSA after WNV vaccination were selected and used in a western blot. These horses are classified as positive responders. Sera from horses in previous that did not show an increased induction of IgE to BSA after WNV vaccination were selected and used as negative responders.

**MATERIALS & METHODS**

**Western Blot Protocol**

1) **Sample Preparation**

- 40 uL, DOT
- 50 uL of Novex by Life Technologies NuPAGE LDS Sample Buffer 4x 3 uL of Fetal Bovine Serum 107 µL De-ionized Water

Combine all components in an eppendorf tube. Heat at 90-100 degrees Celsius for 10 min, then centrifuge for 1 min.

2) **Gel Electrophoresis**

Insert NuPAGE 4-12% Bis-Tris ZOOM Gel into the Novex X Cell Surelock running tank with comb removed. Fill with NuPAGE MOPS Running Buffer. Using a 1000uL pipette, wash gel wells with running buffer from the tank. With a 200uL pipette, load the sample into the large well. Using a 10 uL pipette, load the protein standard marker 10uL BioRad Precision Plus Protein Kalediscope or 5-9UL Life Technologies MagicMark XP Western Protein Standard Protocol. Run the gel at 100-125 V for 1-2 hours.

3) **Transfer of the Protein**

Remove the gel from the running tank and place in a tray filled with Novex by Life Technologies NuPAGE Transfer Buffer. Open the gel case in the transfer buffer. The transfer stack order should be: back case - sponge - blot paper - gel - membrane - sponge - front case. Load the transfer case into the BIORAD Mini Trans-Blot Cell that is filled with transfer buffer. Run the transfer at 20 V overnight in a cold room.

Remove membrane from the transfer case and soak in Sigma Ponceau S Solution. The membrane should always be kept moist with PBS, water, transfer buffer, or milk. On a cutting board, use a razor blade and ruler to cut 2-3 mm width strips. Place strips into Western blot wells.

4) **Blocking the Strips**

Block the strips for 1 hour, rocking, with either Rockland blotTOO Immunoblotting Grade Non-Fat Dry Milk 3% in PBS, or with 1% Casein in PBS. Bands between markers 200-150 were observed, bands between markers 150-250 were observed, and bands above the 250 marker were observed. My original intention was to compare sera from IgE positive horses that had been treated with an immunomodulator (CGG oligodeoxynucleotides) with their pre-treatment serum. However, more consistent definition of IgE bands will be required before this next step is taken.

**OBJECTIVES**

Determine via Western Blot which of the FBS proteins are targets for IgE binding in horses.

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