

The Uterine Mucosal Immune Response in Mares Following Intranasal Vaccination.

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Introduction: Intranasal *Salmonella* vectors show promise in stimulating immunity to various pathogens at mucosal sites throughout the body. An attenuated strain of *Salmonella enteritica* serotype *typhimurium* (*S. typhimurium* MGN 707) expressing the protective protein of *Streptococcus equi* subspecies *zooepidemicus* Moore and Bryans serovar 9 (SzP-MB9) was used to explore the feasibility of using intranasal *Salmonella* vectors to boost equine uterine immunity.

Materials and Methods: Ten reproductively sound Standardbred mares were randomly assigned into vaccinated (n=5) and non-vaccinated (n=5) groups. Mares in the vaccinated group received 2 doses, 2 weeks apart, of 2.5×10^9 CFU of live vaccine intranasally, delivered in 2 ml of normal saline via a hand-pumped nebulizer. Serum, nasal and uterine washings were collected from both groups before and approximately 6 and 12 weeks after vaccination. *Salmonella* cultures were performed on samples from pastures and drinking water of both groups before and after vaccination. During the week after each vaccination, mares in the vaccinated group received daily physical examinations and fresh fecal samples were collected rectally for *Salmonella* culture. Immune responses were assessed by Enzyme-Linked-Immunosorbent-Assay (ELISA). Plates were coated with lipopolysaccharide (LPS) of *S. typhimurium* and purified recombinant SzP-MB9 separately. Serum responses were detected using protein-G conjugated to horseradish peroxidase (HRP); nasal and uterine responses were detected by anti-equine-IgA HRP conjugate. Ortho-phenylene-diamine (OPD) was used as substrate for color development. Optical density was measured by spectrophotometer, and results analyzed by one-way, analysis of variance.

Results: *Salmonella* was not recovered from any of the samples submitted for culture. Vaccinated horses remained normal on physical examination, with no evidence of pyrexia, inappetance or diarrhea. Significant ($P < 0.05$) anti-LPS responses were seen post vaccination in serum, nasal, and uterine washings of vaccinated but not control horses. Significant anti-SzP-MB9 responses were seen in nasal washings of vaccinated animals, but not in serum or uterine washings. Pre-existing levels of antibodies to SzP-MB9 were high in serum of all vaccinated horses. Increased post-vaccination uterine response to SzP-MB9 appeared in 3 horses with low pre-vaccination anti-SzP-MB9 IgA in uterine washings. In the other 2 horses, which had high pre-vaccination anti-SzP-MB9 IgA, post-vaccination uterine responses were slightly negative.

Discussion and Conclusion: These data confirm that intranasal vaccination with attenuated *S. typhimurium* may safely stimulate nasal and uterine immune responses in horses. However, pre-existing antibodies in uterine fluid may have affected responses to SzP-MB9.

Key words: Equine Vaccination *Salmonella* Uterus Intranasal