

IMMUNE RESPONSES TO DIFFERENT STRAINS OF *STREPTOCOCCUS ZOOEPIDEMICUS* INTRAUTERINELY INOCULATED IN MARES

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Introduction: *Streptococcus zooepidemicus* (*S zoo*), a common commensal of the equine genital and respiratory tracts, can manifest itself in a variety of economically significant diseases, including pneumonia, placentitis, and post-breeding endometritis. The objective of this study was to determine if intrauterinely inoculated *S zoo* provoked an immune response in mares that was cross reactive with other *S zoo* strains.

Materials and Methods: The study took place over the ovulatory seasons of 2000 and 2001. In 2000, 4 reproductively healthy Standardbred mares, aged 2 to 9, were intrauterinely inoculated between day 3 and 5 post ovulation with 10 billion CFU of a uterine isolate of *S zoo* (CA-1) on three successive estrous (cycles 1, 2 and 3). They were then inoculated with a second isolate of *S zoo* (10323), on the next 3 cycles (cycles 4, 5 and 6). Estrous cycles were monitored by transrectal ultrasonography. On day 4 post inoculation all mares received 5 mg PGF 2 alpha IM to hasten their return to the next estrous cycle. Serum samples were collected from each horse immediately prior to each inoculation, and at 2 weeks after the final inoculation on cycle 6. At the time of each serum collection, a heparinized blood sample was collected to determine the ability of each horse's blood to kill three different isolates of *S zoo* (CA-1, 10323, and UF-1). Immune recognition of these isolates was also assessed by immunoblotting as follows. Streptococci were washed in 0.9% NaCl, then subjected to acid extraction at 95 C for 15 minutes in 0.85% NaCl adjusted to pH 2.0 with 1 N HCl, electrophoresed in 12% polyacrylamide gel and electrophoretically transferred to nitrocellulose. Binding of these acid-extracted streptococcal antigens by antibodies in mare sera was determined in immunoblots using protein G conjugated to horseradish peroxidase. In 2001, the procedure was repeated using 4 different mares, and the order of inoculation reversed (10323 on cycles 1, 2 and 3 and CA-1 on cycles 4, 5 and 6).

Results and Discussion: In 2000, all mares developed the ability to kill CA-1 in their blood, but not 10323. Similarly, strong responses were detected against CA-1 but not 10323 in immunoblots. In 2001, all mares eventually developed the ability to kill both isolates, though the response to 10323 was delayed in one mare. Immunoblots showed stronger responses to 10323 than in the previous year while responses to CA-1 were less marked. For both isolates, the antigen with the strongest strain specificity was among the smaller proteins extracted, with a molecular weight of approximately 20 kD. No mares killed UF-1 in their blood, or displayed recognition of UF-1 in immunoblots. These results confirm the antigenic differences among different strains of *S zoo*, and do not indicate a boosting of serum immune response when a second strain of *S zoo* is inoculated.

Key Words: Equine Uterus Endometritis *Streptococcus zooepidemicus*