

# Variance in Response to Vaccination of Dairy Cows with Recombinant *Arcanobacterium pyogenes* pyolysin, by Breed

S. Peder Cuneo, DVM, MS; Dusti J. Pruna, BS; Stephen J. Billington, PhD; J. Glenn Songer, PhD; B. Helen Jost, PhD

Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ 85705

## Introduction

*Arcanobacterium pyogenes* is a common inhabitant of the mucous membranes of dairy cattle. This organism is an opportunistic pathogen, associated with suppurative infections of the skin, joints and visceral organs, as well as abortion and mastitis. *A. pyogenes* secretes pyolysin, a hemolytic exotoxin which is both an important virulence factor and a host protective antigen. The object of this study was to determine whether vaccination of dairy cows with recombinant pyolysin would result in production of a neutralizing antibody response against pyolysin, with the ultimate goal of designing a vaccine against *A. pyogenes* mastitis. Brown Swiss and Holstein cows were included in the vaccination trial, to determine whether breed influenced response to the vaccine.

## Materials and Methods

Twenty-seven Holstein and 20 Brown Swiss mature cows from the University of Arizona Dairy Research Center were divided into control and vaccinate groups. At dry-off and on entry to the pre-fresh pen, cows in the vaccinate group received two subcutaneous immunizations consisting of 250 mg recombinant pyolysin in 20% aluminum hydroxide as an adjuvant. Recombinant pyolysin was expressed in *Escherichia coli* and purified to >95% homogeneity by metal affinity chromatography. Control animals were mock-immunized with saline. Blood was collected prior to each immunization, and

approximately 5-6 days post-calving. The serum antibody titer to pyolysin was determined using a hemolysis neutralization assay, and the average titers of the initial and final bleeds from each group were determined.

## Results

Both breeds had pre-existing neutralizing titers to *A. pyogenes* pyolysin. In both the Brown Swiss and Holstein control groups, the levels of pyolysin neutralizing antibodies did not significantly change over the trial period, with initial titers of 97 and 84.4, and final titers of 84.4 and 97, respectively. Variation was observed between breeds in the vaccination group. The initial titer from the Brown Swiss vaccinate group was 107.6, which increased to 181, an approximate 1.7-fold increase. Cows in the Holstein vaccinate group developed a greater response to immunization with recombinant pyolysin, as the initial titer of 78.8 increased approximately 3.2-fold to 256. Effect of breed on the incidence of clinical *A. pyogenes* mastitis in cows at the University of Arizona Dairy Research Center will also be discussed.

## Conclusions

These data indicate that while vaccination with recombinant pyolysin can induce neutralizing antibodies in dairy cows, breed influences the outcome. Holstein cows demonstrated a greater serological response to immunization with recombinant pyolysin compared with Brown Swiss cows.