Application of Siderophore Receptor Proteins and Porins (SRP®) Technology for Controlling *Klebsiella* Mastitis in a Commercial Dairy Herd

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Nearly all bacteria require iron to grow and iron acquisition systems are known to be conserved across many pathogens. To facilitate iron uptake, bacteria release highly specific transport systems, called siderophores that have high affinity for ferric iron and have the ability to strip it away from host binding proteins. For each type of siderophore, bacteria express a complimentary outer membrane siderophore receptor protein (SRP®) that facilitates transport of the iron-siderophore complex through the bacterial outer membrane. For the production of a *Klebsiella* SRP vaccine for reduction of bovine mastitis, *Klebsiella* is grown under iron restriction in large scale fermentation, mimicking the low iron environment encountered during natural infection of the host. This causes the bacteria to express iron acquisition systems, which scavenge iron during fermentation. The bacterial culture is then harvested and processed through an extensive purification process, yielding a highly purified and concentrated preparation of iron regulated outer membrane siderophore receptors and porin proteins.

The SRP's of the family *Enterobacteriacae* have been shown to be highly conserved, making them novel vaccine targets. SRP proteins of *Salmonella* Newport have been successfully exploited as vaccine antigens to control disease caused by *Salmonella* Newport in the dairy industry for over a decade. This same technology has now been developed to combat *Klebsiella* mastitis. The objective of this study was to evaluate efficacy of a *Klebsiella pneumoniae* bacterial extract (Kleb-SRP) vaccine based on SRP technology, to reduce *Klebsiella* mastitis in a commercial dairy herd.

The study was conducted at the Iowa State University (ISU) Dairy in Ames, Iowa. The ISU Dairy consists of approximately 400 milking cows, plus heifers and dry cows. This herd was chosen due to an ongoing *Klebsiella* mastitis problem, which had persisted despite using multiple doses of *E. coli* J5 core antigen vaccine annually. Cows were randomized to receive the Kleb-SRP vaccine or a placebo vaccine containing adjuvant only. All cows in both groups continued to receive the standard E. coli J5 vaccine throughout the study. The study was double-blinded and animals were blocked so groups were approximately equally distributed based on their lactation (1st, 2nd, and 3+), pre-study somatic cell count (SCC), and days in milk (DIM).

A whole-herd enrollment was conducted at study onset by vaccinating cows subcutaneously with their assigned treatment. In all, 569 individuals were analyzed in the study. Cows within 5 weeks of parturition were not vaccinated at initial enrollment, but instead vaccinated 2 weeks after parturition. Vaccinations were repeated after three weeks. Each week throughout the study, lactating cows and new heifers achieving ~217 days carrying calf (DCC) were vaccinated and followed with a repeat dose 3 weeks later. For each cow, the eligible observation period began 2 weeks after the second vaccination and was continued for the first 90 days in milk. Cows were monitored for clinical mastitis, and if confirmed positive, a milk sample was submitted to the ISU Veterinary Diagnostic Laboratory to determine the causative agent. Bacterial identification was confirmed by MALDI. Cows were then treated according to standard protocols for the dairy. Data on milk production was recorded daily, and SCC was determined by DHIA on approximately 5 week intervals. The study was approximately 10 months in duration.

The prevalence and incidence of *Klebsiella* mastitis was significantly reduced in Kleb-SRP vaccinated cows compared to placebo vaccinated controls. Prevalence, the portion of individuals that tested positive during the study, was reduced by 71% in vaccinates compared to controls (Figure 1. Controls: 14 of 225, Vaccinates: 4 of 225; Prevented Fraction 0.7143; 95% CI: 0.1453 to 0.9045; p = 0.0171). Incidence, which accounts for multiple infections in a single individual, was reduced by 76% (Controls: 16 incidents, Vaccinates: 4 incidents; Prevented Fraction 0.7594; 95% CI: 0.2804 to 0.9195; p = 0.0056).

Milk Production increased in Kleb-SRP vaccinated cows by 2.0 pounds per cow per day compared to placebo cows (Figure 2. $p < 0.001$). The largest differences in milk production occurred during months 4, 5 and 6 of the study (summer months). Further, incidence of elevated SCC measures (over 200,000 cells) were significantly reduced in Kleb-SRP vaccinated compared to placebo cows (prevented fraction 0.5357; 95% CI 0.3196 to 0.6832, $p < 0.001$). Overall, Kleb-SRP vaccinated cows had a 42% reduction in SCC compared to placebo controls ($p < 0.001$).

Figure 1: Cumulative Cases of Klebsiella Mastitis

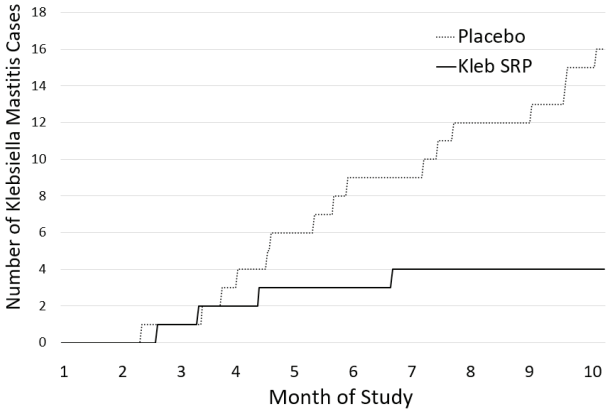
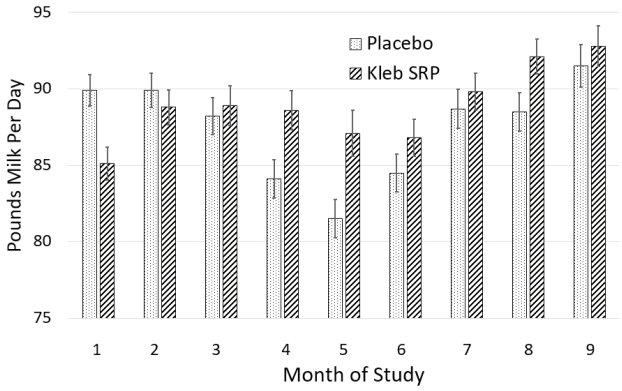


Figure 2: Average Daily Milk Production



Conclusions

Vaccination with a *Klebsiella pneumoniae* vaccine based on the SRP® technology provided statistically significant protection from *Klebsiella* mastitis. The significant increase in milk production and decrease in SCC among Kleb-SRP vaccinated cows compared to controls requires further evaluation, since it cannot be explained solely by the control of clinical mastitis.

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