for culture and SCC was collected immediately before challenge, and 3, 6, 9, 12, 15, 18, 21 and 24 hrs after challenge, as well as at each milking every 12 hrs until eight days post-challenge. For all post-challenge milk cultures, if *E. coli* was isolated, quantification of bacterial shedding in cfu/ml of milk was performed. All frozen serum and milk samples were tested using an ELISA method for IgG1, IgG2 and IgM specific for J-5 *E. coli* core antigen at the Immunology and Immunogenetics Laboratory at Michigan State University. Samples were titer diluted until the O.D. was <100. There were two replications using duplicate sub-samples for each of four dilutions for each antibody type. All data were entered into Excel, and statistical analyses were performed using SAS.

Results

Quarters selected for challenge had no infections with major pathogens, and SCC was 87,000/ml or less in all eight quarters selected for challenge. Cows were challenged between eight and 16 days in milk (DIM), with the median being 13. None of the eight cows developed severe clinical mastitis or systemic disease signs. At many time points during the seven days post-challenge, the vaccinates had significantly higher milk production and lower SCC than the controls ($P < .05$, ANOVA). Preliminary analysis showed that total milk production for seven days following challenge was significantly higher for cows vaccinated with J-5, and they had higher blood levels of (J-5 strain-specific) IgM at calving and pre-challenge. Vaccinates also had significantly higher milk IgG levels pre-challenge. Further analyses, including milk antibodies and possible implications for the mechanisms of J-5 bacterin protection, will be discussed.

Long-term Persistence of Genetic Types of Mastitis-causing *Staphylococcus aureus* on Three Dairy Herds

R. Lyman, *BA*[1]; **C. George,** BS[2]; **W. Kloos,** *PhD*[2]; **C. Spooner,** *DVM*[1]; **K. Anderson,** *DVM, PhD*[1]
[1]*College of Veterinary Medicine, NC State University, Raleigh, NC 27606*
[2]*College of Agriculture and Life Sciences, NC State University, Raleigh, NC 27695*

Introduction

Staphylococcus aureus remains a significant cause of mastitis in the dairy industry. It has been recommended that control of *S. aureus* mastitis be focused on the specific genetic types of *S. aureus* that most commonly cause the mastitis. Genetic types can be defined using molecular techniques, including pulsed field gel electrophoresis (PFGE). There have been comparatively few studies looking at within-herd persistence over time of *S. aureus* genetic types as causes of mastitis. Most have dealt with very few isolates or comparatively short periods of time. The objective of this investigation was to examine PFGE band patterns (EPs) from bovine mastitis-causing *S. aureus* isolates from three related herds over the course of 15 years to determine if any patterns persisted long-term.

Materials and Methods

In 1998, a dairy herd (A) in North Carolina experienced an increase in bulk-tank milk somatic cell counts to levels above 1 million cells/ml, with decreased milk production. Bulk-tank milk analysis found 3000 colonies/ml of *S. aureus*. Culturing of milk samples indicated that approximately one-third of herd cows had *S. aureus* intramammary infections.

In 2000, a second and related dairy herd (B) showed increased somatic cell counts and decreased production. Approximately one-half of the lactating herd was infected with *S. aureus* mastitis. These two herds were related by management and movement of cows.

Individual cow and bulk-tank milk samples have been collected at intervals until the present at both dairies as well as at a third, related dairy (C). Additional samples had been sporadically cultured from 1988 to 1998 and *S. aureus* isolates saved. Milk samples or isolates were frozen at -70 to -80°C (-94 to –112° F). Identification of *S. aureus* was according to National Mastitis Council procedures.

PFGE was performed on one or more samples from all *S. aureus*-positive cows using previously described methods. Only one isolate per cow was included unless more than one EP was found in a single cow. A total of

305 *S. aureus* isolates were identified for use in this study.

An EP was considered to persist long-term if it was found in samples collected 10 or more years apart.

Results and Discussion

Forty EPs of *S. aureus* were identified between 1988 and the present in the three herds studied. Of these, seven have persisted long-term in the combined herds. One persistent EP was common to all three herds. These results indicate that some EPs of *S. aureus* can persist long-term in some herds. Determining the bacterial properties that allow long-term persistence may lead to enhanced methods to manage *S.aureus* mastitis.

Re-thinking Clinical Mastitis Therapy

J.L. Hess, L.M. Neuder, P.M. Sears
Department of Large Animal Clinical Science, Michigan State University, East Lansing, MI 48824

Introduction

The course of treatment for clinical mastitis varies greatly from farm to farm. In most large herds (500 cows or greater), a cow is separated from the production herd when clinical mastitis is detected by abnormal milk. Abnormal milk is an indication of a problem that can occur with or without swelling and fever. When clinical mastitis is brought to the attention of the herdsman, he must make the decision to move the cow and start treatment. Unfortunately, this is often the point where treatment decisions are based on experience and opinions, rather than good scientific guidelines and protocols. In order to implement an effective clinical protocol, it is useful to determine the cause of infection and implement a treatment protocol that is specific for that type of infection. A good treatment protocol can reduce antibiotic use with fewer days of unsaleable milk.

Materials and Methods

A large commercial dairy located in central Michigan, milking 3200 cows twice a day with excellent facilities, was enrolled in a clinical mastitis treatment project. This study took place from October 2001 to May 2002.

Culturing Clinical Cows. Milk samples from clinical quarters were collected aseptically and cultured on-farm. The milk sample was placed on a standard blood agar with 1% esculin and MacConkey agar plates. All streptococcal organisms were transferred to a CAMP test to identify the presence of *Streptococcus agalactiae*. *Staphylococcus aureus* was confirmed using a coagulase test. Coliforms were isolated on MacConkey agar and *Eschericha coli* and *Klebsiella* sp were identified by lactose fermentation, bile salt precipitation and other colony characteristics.

Treatment Protocol. Starting in October of 2001, clinical cases were not treated until after the culture results were entered on the cow's record in Dairy Comp 305. If either *E. coli* or *Klebsiella* were identified on culture, the cow was marked "NO TREAT" and the quarter was monitored. All others were marked "TREAT" and started on antibiotic therapy. In early February, half of the cows that cultured "no growth" were removed from the treatment group, while the other half continued the routine treatment protocol. The groups were compared for return to normal milk, days out of production and quarter loss.

Results

The majority of clinical cases occurred in the first 100 days of lactation, with peaks at 25 days and 75 days for gram-negative bacteria (*E. coli* and *Klebsiella* sp) infections. The greatest number (28%) of gram-positive bacteria infections (*Strep* sp and *Staph* sp) were cultured in the first 25 days, with the remaining infections occurring throughout lactation.

In February, when the treatment protocol was changed to limit antibiotic therapy to cows that were culture-positive for gram-positive bacteria, the number of cows requiring intramammary antibiotics was reduced 80%. Fifty-five percent of the clinical quarters cultured "no growth", and 25% cultured gram-negative bacteria which did not require intramammary antibiotics. Very few of the clinical cases were ill or had a fever that required immediate attention. When treatment was withheld for 24 hours awaiting the culture results, most clinical signs had resolved and the gram-negative and "no-growth" quarters did not require treatment. Cows assigned to treatment in the "no-growth" category did not return to normal milk quicker and did not have fewer quarters lost.