

**Table 1.** Reproductive performance for 389 cows with and without subclinical endometritis

Parameter	Polymorphnuclear Neutrophils	
	<5%	>5%
Number of Cows	229 (58.9%)	160 (41.1%)
Cows after VWP	207	132
Cows inseminated	191/207 (92.3%)	114/132 (86.4%)
Days to 1 <sup>st</sup> AI	89.5	88.9
1 <sup>st</sup> AI conception rate	54.4% (98/180)	46.1% (47/102)
Cows pregnant	77.8 %	65.9 %
Days open	112.0	115.9
Cows culled	13	12

VWP= voluntary waiting period

AI = artificial insemination

## J-5 Bacterin Protection Against *Escherichia coli* Intramammary Challenge and Association with Milk and Blood Levels of J-5-specific Antibodies

**David J. Wilson, DVM, MS<sup>1</sup>; Bonnie A. Mallard, BSc, MSc, PhD<sup>2</sup>; Jeanne L. Burton, BSc, MSc, PhD<sup>3</sup>; Ynte H. Schukken, DVM, MS, PhD<sup>1</sup>**

<sup>1</sup>Cornell University, Ithaca, NY

<sup>2</sup>University of Guelph, Guleph, Ontario, CA

<sup>3</sup>Michigan State University, East Lansing, MI

### Introduction

This was an *Escherichia coli* intramammary infection challenge trial evaluating a J-5 coliform mastitis commercial vaccine. Antibodies specific for J-5 strain of *E. coli* (IgG1, IgG2 and IgM) were measured in cows' milk and blood in response to vaccination and in response to IMM challenge with *E. coli*. Several outcome measures of mastitis severity and milk production response were compared among vaccinates and controls.

### Materials and Methods

Eight Holstein dairy cows with at least one previous lactation were studied. Cows had records of low somatic cell count (SCC), no major episodes of any disease, and were close to drying-off when the study began. Duplicate aseptic quarter milk samples were cultured from all eight cows just before drying-off, approximately 50 days before they were due to calve again. Four cows were controls and four were vaccinates. J-5 bacterin was administered subcutaneously in the supramammary region just before cows were dry, and

again four weeks later, during the mid-dry period. At mid-dry period, approximately three weeks before calving, blood samples were collected and aliquots of serum were frozen at  $-80^{\circ}\text{C}$  ( $-112^{\circ}\text{F}$ ).

After calving, all quarters were milked individually and all quarter milk weights were recorded. Aseptic milk samples were cultured from each quarter seven days pre-challenge and two days pre-challenge for intramammary infection (IMI), and quarter milk was also tested for SCC seven days, two days, and one day before challenge to aid in selecting the challenge quarter.

Intramammary challenge solution was with an *E. coli* strain (1000 cfu) that had been used in previously reported mastitis challenge trials. After calving, both quarter milk and blood samples were collected 12 hours before intramammary challenge; and 12 and 24 hours after challenge. Milk was cultured and aliquots of supernatant were collected and frozen at  $-80^{\circ}\text{C}$  ( $-112^{\circ}\text{F}$ ).

Following the intramammary challenge, milk weights were recorded at all milkings from the challenged quarter, the contralateral quarter, and the composite milking of the other two quarters. For the challenged quarter only, aseptic milk (duplicate samples)

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<sup>1</sup>; C. George, BS<sup>2</sup>; W. Kloos, PhD<sup>2</sup>; C. Spooner, DVM<sup>1</sup>; K. Anderson, DVM, PhD<sup>1</sup>

<sup>1</sup>College of Veterinary Medicine, NC State University, Raleigh, NC 27606

<sup>2</sup>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