

# Variation in Weekly Shedding Pattern of *Staphylococcus aureus* in Naturally Occurring Intramammary Infections

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## Introduction

Despite improvements in management, prevention, and treatment, mastitis accounts for 26% of morbidity on US dairies, making it the most prevalent and costly disease of dairy cattle. In total, it is estimated that 70-80% of this loss is due to subclinical intramammary infections (IMI) such as *Staphylococcus aureus* (SA). The most prevalent contagious mastitis pathogen in the United States, SA was detected in 43% of bulk tanks examined. The control of SA is contingent on accurate diagnosis of IMI, yet there remains no definitive standard for the diagnosis of a SA IMI. Studies following cows experimentally infected with the Newbould SA strain concluded that SA was shed in a cyclical pattern and that consecutive samples were necessary to accurately diagnosis a SA IMI. Since then, new technologies, such as pulse field gel electrophoresis (PFGE) used to compare strain relatedness, have brought into question the usefulness of research based on experimental SA infections. The goal of this study was to evaluate bacterial shedding patterns of SA, specifically the influence of clonal relatedness of SA on the shedding patterns of bacteria in naturally occurring SA infections.

## Materials and Methods

Foremilk samples were collected weekly (according to NMC guidelines) from quarters in 22 lactating cows (29 quarters) at two dairies identified with SA IMI in the previous lactation or within the first seven days of the current lactation, for 26 to 44 weeks. Milk samples that were culture-negative for SA using a 0.01 mL inoculum from quarters that had been confirmed SA-positive were thawed a second time and cultured using a larger volume of milk (0.1 mL). A sample was considered culture-positive for SA with  $\geq 1$  cfu/0.01 mL. Longitudinal shedding patterns of SA over an eight-week window (bandwidth=0.18) were examined using

the lowest smoother application (STATA). The effect of strain type on ln (cfu) was examined using a linear mixed model (SAS).

## Results

Using the 0.01 mL inoculum, 914 of 1,070 samples (85%) were culture-positive ( $\geq 1$  cfu/0.1 mL). Using a 0.1 mL inoculum, 1,011 (95%) of the samples were culture-positive ( $\geq 1$  cfu/0.1 mL). There was no significant difference in the detection of SA between PFGE pulsotypes when using either the 0.01 mL inoculum ( $P=0.076$ ) or the 0.1 mL inoculum ( $P=0.1$ ). Of the 59 culture-negative samples using 0.1 mL, three quarters (in two heifers) accounted for 68%. While there appears to be a large difference in the amount of shedding from quarters infected with different pulsotypes, there was no significant difference ( $P=0.1$ ) in the amount of shedding between quarters infected with isolates grouped in pulsotype 1 or 2. There was no consistent pattern of shedding identified between or within cows, with great variation noted between quarters within the same cow. There was a significant difference in linear score and test-day linear score between quarters infected with isolates in PFGE pulsotype 1 and 2.

## Significance

Contrary to previous reports of intermittent shedding, while the level of shedding in these naturally occurring infections varied, 95% of the samples were culture-positive using a larger, 0.1 mL, inoculum. Results demonstrated a remarkably consistent ability to detect SA infections when a larger inoculum was used. Given the dramatic differences in shedding within cows, future research examining whether such variation is primarily due to the biology of SA and the individual cow or due primarily to methodology will aid in developing best management practices to control SA IMI.