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

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Introduction

The cost of mastitis has been calculated at 6% of the value of production; at 2007 prices that is upwards of 2.1 billion dollars. It is estimated that 70-80% of this loss is due to subclinical intramammary infections (IMI) caused by organisms such as *Staphylococcus aureus* (SA). The control of SA is contingent on accurate diagnosis of IMI, yet currently there exists no standard for the diagnosis of SA IMI. As a result, comparisons between published works are difficult. In addition, the shedding of SA from infected quarters has been described as "intermittent", resulting in recommendations for the diagnosis of SA IMI that are cumbersome and cost-prohibitive in veterinary practice and field research. The goal of this study was to describe shedding patterns of naturally occurring SA IMI over an extended period of time and to provide a reasonable foundation upon which to determine appropriate diagnostic criteria for SA IMI. The effect of PFGE pulsotype on shedding was also examined.

Materials and Methods

Milk samples were collected according to NMC guidelines from five multiparous cows (seven quarters) and two primiparous cows (two quarters). Milk samples were collected for 21 consecutive days, three times throughout the lactation (63 days total), frozen immediately and kept frozen for up to 22 days, and thawed at room temperature for microbiological examination. The initial culture procedure employed standard NMC guidelines. Colony counts were recorded up to 100. A SA IMI was defined as a quarter culture-positive with ≥1 cfu of SA/0.01 mL within the first three days of the first 21-day sample period. Individual quarter samples with ≥1 cfu of SA were considered positive. The milk samples were kept frozen and thawed again at a later date, and re-examined using an entire plate per sample recording cfu up to 1,000 cfu/0.01 mL. A representative isolate from each quarter was submitted for pulse field gel electrophoresis (PFGE), with PFGE clusters evaluated at 80% similarity. Initial exploratory analysis was done using STATA (v.10). Longitudinal shedding patterns of SA were examined with locally weighted regression (lowess, STATA v. 10). The effect of PFGE pulsotype on the amount of shedding was examined using a linear mixed model with heterogeneous autoregressive correlation structure (PROC MIXED, SAS 9.2).

Results

A total of 397 milk samples from SA-infected quarters were collected for microbiological examination and 388 (97.7%) of them were culture-positive (≥ 1 cfu/0.01 mL) for SA. A total of 393 milk samples from nine quarters (seven cows) were included in the analysis. Only four of the original seven cows remained for three sample periods, for a total of 63 days. Two quarters (one cow) were followed for 42 days, and three quarters (two cows) were followed for 21 days. Results from the second plating (counted up to 1000 cfu) were used in the analysis. On the second culture 97.5% of the samples (383 of 393) were culture-positive (≥ 1 cfu). Examination of the shedding pattern revealed the consistent presence of SA, although at varied levels, in addition to different levels of shedding between quarters in the same cow. The longitudinal shedding patterns of SA over the 21-day sample periods revealed no predominant cyclic shedding patterns. Accounting for the effect of sample day, samples collected from quarters infected with SA in pulsotype I had a ln(cfu) 1.5 times greater than those in pulsotype II (P=0.007).

Significance

Contrary to previous reports describing the "intermittent" shedding of SA, our study found that, although daily shedding was variable between and within cows, SA was consistently recovered from quarters infected with SA. While strain type may have an effect on the overall amount of bacterial shedding, our study would support that a single sample, culture-positive with one cfu, is sufficient to diagnose a SA IMI. Future studies examining naturally infected quarters over longer periods may further aid in developing more coherent prerequisites of infections.