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Intramammary infections with different non-*aureus* staphylococci in dairy cows

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ABSTRACT

Subclinical mastitis causes an increase in milk somatic cell count (SCC) and can lead to reduced milk production and early culling. In many countries, non-*aureus* staphylococci (NAS) is the most common bacterial finding in subclinical mastitis of dairy cows. New methodology makes it possible to identify NAS species, but knowledge about the epidemiology is limited. The objective of this project was to improve advisory services for mastitis control by investigating associations between NAS and SCC, milk production, and persistence of intramammary infections (IMI). Farmers who had sent milk samples to the Swedish National Veterinary Institute (Uppsala, Sweden) were asked to participate if NAS was identified in the samples. Participating farmers were asked to resample all udder quarters of the cow once within 1 mo. Regression models were used to investigate associations between NAS and cow factors, udder quarter California mastitis test and SCC, and persistence of IMI. Associations with cow composite milk yield and SCC were also investigated. In total, 671 cows from 201 herds were enrolled in the study, and 19 NAS species were identified, of which the 4 most common were *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus chromogenes*, and *Staphylococcus haemolyticus*. Persistent IMI was more common in udder quarters with *Staphylococcus hyicus* and *S. simulans* and less common in those with *Staphylococcus saprophyticus* IMI. β-Lactamase production by the different NAS species varied from 0 to 100%. There was a significant association between NAS species and California mastitis test and SCC of udder quarters, and this varied depending on parity. The cow composite milk SCC at the test milking before the initial sample was taken differed significantly with NAS species, but not at the subsequent test milking. Milk yield—at the test milking before or after the initial sample—did not differ significantly for NAS species. There were no significant associations between milk yield or SCC and persistent NAS IMI. In conclusion, the NAS species affects SCC and persistent IMI differently but not milk yield.

Key words: mastitis, coagulase-negative staphylococci, persistence, antimicrobial susceptibility

INTRODUCTION

Mastitis in dairy cows causes financial losses for the farmers (Halasa et al., 2007), reduces milk production and quality (Hagnestam et al., 2007; Hagnestam-Nielsen et al., 2009; Forsbäck et al., 2010), and has a negative effect on animal welfare. The financial losses are mainly due to the decreased milk production and quality (Hagnestam et al., 2007; Dürr et al., 2008), treatment costs, increased workload for the farmers, and increased culling (Halasa et al., 2007). In Sweden, about 35% of first-parity cows and approximately 20% of older cows suffer from new IMI each year (VäxaSverige, 2016). The most common cause of these infections is *Staphylococcus aureus* followed by non-*aureus* staphylococci (**NAS**; VäxaSverige, 2016). In subclinical mastitis, NAS is also the second most frequent finding after *S. aureus* (Persson et al., 2011) in Swedish dairy cows. In other countries (e.g., Finland, the Netherlands, Germany, and South Africa), NAS is the most prevalent finding in mastitis (Pitkälä et al., 2004; Tenhagen et al., 2006; Petzer et al., 2009; Sampimon et al., 2009).

Non-*aureus* staphylococci are a heterogeneous group of bacterial species. More than 50 species and subspecies have been identified (LPSN, 2017), of which 20 are commonly found in cow milk (Vanderhaeghen et al., 2015).The main reason for not differentiating the NAS previously has been lack of reliable and efficient methods. Phenotypic identification with commercial kits has shown limited typeability and accuracy (Thorberg and Brändström, 2000; Taponen et al., 2006; Capurro,

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2009). Techniques based on molecular identification have mostly been available only in research laboratories, but matrix-assisted laser desorption/ionization time-of-flight (**MALDI-ToF**) MS analysis is becoming more common. At the National Veterinary Institute in Uppsala, Sweden, MALDI-ToF MS has been used in routine analysis of milk samples since 2013. With this technology, most NAS isolates can be identified at species level with high accuracy (Cameron et al., 2017; Pizauro et al., 2017) within minutes and at a low cost per isolate.

Previously, NAS was believed to mainly cause subclinical or sometimes mild clinical mastitis, but more recent research shows that IMI with NAS can cause chronic mastitis, damage udder tissue, and decrease milk production (Taponen et al., 2007; Thorberg et al., 2009). Less is known about how the specific NAS species affects udder health (Sampimon et al., 2009; Thorberg et al., 2009; Supré et al., 2011). Some virulence genes are present for some species but not for others (Martins et al., 2017), and their susceptibility to antimicrobials varies (Persson Waller et al., 2011; Raspanti et al., 2016; Taponen et al., 2016). Moreover, the knowledge about the distribution and epidemiology of NAS species in Sweden is sparse. Hence, the objective of the present study was to investigate the most commonly found NAS IMI of Swedish dairy cows with suspected subclinical mastitis and how the species are associated with cow factors, antimicrobial susceptibility (β-lactamase production), SCC, milk yield, and persistence of IMI.

MATERIALS AND METHODS

Recruitment of Farmers and Herds

All dairy farmers who submitted quarter milk samples because of suspected subclinical mastitis to the National Veterinary Institute (Uppsala, Sweden) between September 2014 and May 2015 were contacted by phone to join this cross-sectional study if NAS was identified in the routine analysis. Participating farmers had to be affiliated with the Swedish Official Milk Recording Scheme (**SOMRS**), and the cow should not have been treated with antimicrobials between sampling and recruitment. Participating farmers were asked to resample all udder quarters of the cows from which the initial sample came within 2 wk after recruitment. The sample sent in before the actual study started is hereafter called the initial sample, and the subsequent resamples are called the follow-up samples. To show the distribution of the participating herds in relation to the density of Swedish dairy herds, a map was created using ArcMap 10.3.10 (Esri Inc., Redlands, CA).

Follow-Up Sampling

Sterile milk tubes, disinfectant, cotton swabs, and a referral for each cow were sent to the participating farmers. Instructions on aseptic milk sampling and sample storage were also included. The farmers were urged to send the samples in a padded postal envelope to the National Veterinary Institute on the same day as sampling.

Laboratory Analyses

All samples containing enough milk were investigated on the day of arrival with the California mastitis test (**CMT**). Moreover, milk aliquots from a randomly selected subsample were analyzed for SCC 2 to 3 d later using the DeLaval cell counter (DeLaval International AB, Tumba, Sweden). Bacteriological analysis of quarter milk samples and MALDI-ToF identification of the NAS species were performed according to the accredited routines at the National Veterinary Institute. Only colonies from quarter milk samples with growth of bacteria in pure culture were investigated. Criteria for species identification were as follows: a score of $\geq$2 indicated identification at species level, a score of 1.80 to 1.99 indicated identification at genus level, and a score of $<$1.80 indicated no identification. Species identification was performed using a custom-made database including the Bruker databases no. 5627 and 5989 with addition of custom-made spectrums (**MSP**) for some NAS species (2 *Staphylococcus devriesei* MSP, 3 *Staphylococcus pseudintermedius* MSP, 2 *Staphylococcus rostri* MPS, 1 *Staphylococcus fleuretti* MSP, and 1 *Staphylococcus lentus Puerto Rico* MSP). The genotypic or phenotypic characteristics of the NAS strains were not investigated. β-Lactamase production was evaluated by the clover-leaf method (Franklin and Wierup, 1982). For Swedish conditions, β-lactamase resistance is the most important aspect of antimicrobial resistance of staphylococci; hence, resistance to other antimicrobials was not investigated.

Cow Data

Individual cow data (breed, parity, and day of calving) were obtained from the SOMRS as well as the SCC, milk yield (kg/d), percentage of fat and protein in milk, and concentration of milk urea (mmol/L) for all test milkings during 6 mo pre- to 1 yr postrecruitment.

Statistical Analyses

NAS Distribution in Initial Sample and Associations with Cow Factors. Descriptive statistics