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Prevalence of non-*aureus Staphylococcus* species causing intramammary infections in Canadian dairy herds

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ABSTRACT

Non-*aureus* staphylococci (NAS), the microorganisms most frequently isolated from bovine milk worldwide, are a heterogeneous group of numerous species. To establish their importance as a group, the distribution of individual species needs to be determined. In the present study, NAS intramammary infection (IMI) was defined as a milk sample containing ≥1,000 cfu/mL in pure or mixed culture that was obtained from a cohort of cows assembled by the Canadian Bovine Mastitis Research Network. Overall, 6,213 (6.3%) of 98,233 quarter-milk samples from 5,149 cows and 20,305 udder quarters were associated with an NAS IMI. Of the 6,213 phenotypically identified NAS isolates, 5,509 (89%) were stored by the Canadian Bovine Mastitis Research Network Mastitis Pathogen Collection and characterized using partial sequencing of the *rpoB* housekeeping gene, confirming 5,434 isolates as NAS. Prevalence of each NAS species IMI was estimated using Bayesian models, with presence of a specific NAS species as the outcome. Overall quarter-level NAS IMI prevalence was 26%. The most prevalent species causing IMI were *Staphylococcus chromogenes* (13%), *Staphylococcus simulans* (4%), *Staphylococcus haemolyticus* (3%), *Staphylococcus xylosus* (2%), and *Staphylococcus epidermidis* (1%). The prevalence of NAS IMI as a group was highest in first-parity heifers and was evenly distributed throughout cows in parities ≥2. The IMI prevalence of some species such as *S. chromogenes*, *S. simulans*, and *S. epidermidis* differed among parities. Overall prevalence of NAS IMI was 35% at calving, decreased over the next 10 d, and then gradually increased until the end of lactation. The prevalence of *S. chromogenes*, *Staphylococcus gallinarum*, *Staphylococcus cohnii*, and *Staphylococcus capitis* was highest at calving, whereas the prevalence of *S. chromogenes*, *S. haemolyticus*, *S. xylosus*, and *S. cohnii* increased during lactation. Although the overall prevalence of NAS IMI was similar across barn types, the prevalence of *S. simulans*, *S. xylosus*, *S. cohnii*, *Staphylococcus saprophyticus*, *S. capitis*, and *Staphylococcus arlettae* IMI was higher in tie-stall barns; the prevalence of *S. epidermidis* IMI was lowest; and the prevalence of *S. chromogenes* and *Staphylococcus sciuri* IMI was highest in bedded-pack barns. *Staphylococcus simulans*, *S. epidermidis*, *S. xylosus*, and *S. cohnii* IMI were more prevalent in herds with intermediate to high bulk milk somatic cell count (BMSCC) and *S. haemolyticus* IMI was more prevalent in herds with high BMSCC, whereas other common NAS species IMI were equally prevalent in all 3 BMSCC categories. Distribution of NAS species IMI differed among the 4 regions of Canada. In conclusion, distribution differed considerably among NAS species IMI; therefore, accurate identification (species level) is essential for studying NAS epidemiology.

Key words: dairy, mastitis, intramammary infection, coagulase-negative staphylococci, prevalence

INTRODUCTION

Non-*aureus* staphylococci (**NAS**) have been considered pathogens of minor importance in dairy production, particularly compared with major udder pathogens such as *Staphylococcus aureus*, streptococci, and coliforms. Nevertheless, NAS are the bacteria most

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frequently isolated from udder quarters in all recent North American and European subclinical mastitis surveys (Piepers et al., 2007; Pyorala and Taponen, 2009; Sampimon et al., 2009a; Thorberg et al., 2009; De Vliegher et al., 2012; Dufour et al., 2012). Additionally, because NAS IMI moderately increases SCC, lower acceptable limits for bulk milk SCC (**BMSCC**) have increased the relative importance of NAS IMI (Piepers et al., 2007).

Although the high prevalence of NAS is widely recognized, their importance remains a topic of debate (Oliver and Jayarao, 1997; Piepers et al., 2007; Fox, 2009; Nickerson, 2009; Sampimon et al., 2009a; Schukken et al., 2009). Some authors consider NAS a main cause of subclinical and persistent mastitis (Sampimon et al., 2009a; Fry et al., 2014), whereas others suggest that NAS have a protective effect against major pathogen IMI (Matthews et al., 1990; De Vliegher et al., 2004). Additionally, although milk production was higher in heifers with NAS IMI compared with uninfected heifers (Schukken et al., 2009; Piepers et al., 2010), in other studies no effect on milk production (Tomazi et al., 2015) or decreased milk production associated with NAS IMI (Taponen and Pyörälä, 2009) have also been reported.

Apparently contrasting findings among studies regarding the effect of NAS on udder health and milk production could be the result of regarding the NAS as one group (Woodward et al., 1987, 1988; Matthews et al., 1990). However, NAS are a large and heterogeneous group (Zadoks and Watts, 2009; Vanderhaeghen et al., 2015), and it is known that differences exist among NAS species regarding their interactions with the host and the environment; consequently, they have variable effects on udder health and milk production (Piepers et al., 2009; Vanderhaeghen et al., 2014; Piccart et al., 2016). For example, IMI with *Staphylococcus chromogenes*, *Staphylococcus simulans*, and *Staphylococcus xylosus* have a greater effect on SCC compared with IMI with other species, such as *Staphylococcus cohnii* and *Staphylococcus sciuri* (Taponen et al., 2007; Supré et al., 2011; Fry et al., 2014; De Visscher et al., 2016). Some species, such as *S. chromogenes* and *Staphylococcus epidermidis*, seem to be host adapted, whereas others, such as *Staphylococcus haemolyticus*, act as opportunists (Piessens et al., 2011).

Most studies have included staphylococci species with variable responses to the coagulase test, such as *Staphylococcus agnetis*, in the group of CNS (Taponen et al., 2012). This classification was based on the phenotypic characteristic of *S. aureus* to coagulate plasma, often applied at a time when only *S. aureus* was characterized as pathogenic and the other NAS species were considered minor pathogens (Becker et al., 2014). However, this classification grouped species that are not necessarily phylogenetically related and does not accurately reflect the variability within the genus *Staphylococcus* (Becker et al., 2014; Naushad et al., 2016). Therefore, because some NAS species can vary in their response to the coagulase test, we prefer and propose to use the name NAS for this group of species.

Geometric mean BMSCC and housing of lactating cows differ by geographical region (Barkema et al., 2015). Based on the National Cohort of Dairy Farms study conducted in Canada during 2007 and 2008, Dufour et al. (2012) reported no difference in prevalence of NAS IMI among tie-stall, free-stall, and bedded-pack barns when considering NAS as a single group. However, Olde Riekerink et al. (2008) reported a difference between tie stalls and free stall in incidence of clinical mastitis caused by various NAS species. Perhaps differences in management practices among housing systems affect the prevalence of IMI with specific NAS species.

Within-herd prevalence of IMI with various NAS species is influenced by parity and lactation stage (Sampimon et al., 2009a; De Visscher et al., 2016). *Staphylococcus simulans* and *S. epidermidis* are most commonly isolated from multiparous cows (Taponen and Pyörälä, 2009; Mørk et al., 2012), whereas *S. chromogenes* more frequently causes IMI in heifers. In the latter, the prevalence is usually higher close to calving, but persistency is also reported (Taponen et al., 2007). In that regard, *S. simulans* can persist in the udder for long intervals throughout lactation, whereas prevalence of *S. chromogenes* IMI decreases shortly after calving (Piessens et al., 2011). Comparison of distribution between front and rear quarters provides information about the source of the IMI attributable to a particular species. Although Barkema et al. (1997) isolated NAS more frequently in rear quarters than in front quarters, De Visscher et al. (2016) did not report a species-specific NAS quarter distribution. There are apparently no North American data on parity and DIM distribution of NAS causing IMI in bovine udder quarters.

A large field cohort study was conducted during 2007 and 2008 by the Canadian Bovine Mastitis Research Network (**CBMRN**). Data and isolates from this prospective study have enabled further investigation of the relevance of NAS species for the dairy industry. The first objective of the current study was to determine the prevalence and distribution of NAS species causing bovine IMI on Canadian dairy farms. The second objective was to evaluate potential associations of species-specific NAS IMI with herd characteristics (region, BMSCC, and barn type) as well as cow characteristics (parity, DIM, and quarter location).