J. Dairy Sci. 97:5275–5293
http://dx.doi.org/10.3168/jds.2013-7775

Invited review: Effect, persistence, and virulence of coagulase-negative *Staphylococcus* species associated with ruminant udder health

W. Vanderhaeghen,* S. Piepers,* F. Leroy,† E. Van Coillie,‡ F. Haesebrouck,§ and S. De Vliegher*[1]
*M-Team & Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
†Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
‡Technology and Food Science Unit, Institute for Agricultural and Fisheries Research (ILVO), Brusselsesteenweg 370, 9090 Melle, Belgium
§Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ABSTRACT

The aim of this review is to assess the effect of coagulase-negative staphylococci (CNS) species on udder health and milk yield in ruminants, and to evaluate the capacity of CNS to cause persistent intramammary infections (IMI). Furthermore, the literature on factors suspected of playing a role in the pathogenicity of IMI-associated CNS, such as biofilm formation and the presence of various putative virulence genes, is discussed. The focus is on the 5 CNS species that have been most frequently identified as causing bovine IMI using reliable molecular identification methods (*Staphylococcus chromogenes*, *Staphylococcus simulans*, *Staphylococcus haemolyticus*, *Staphylococcus xylosus*, and *Staphylococcus epidermidis*). Although the effect on somatic cell count and milk production is accepted to be generally limited or nonexistent for CNS as a group, indications are that the typical effects differ between CNS species and perhaps even strains. It has also become clear that many CNS species can cause persistent IMI, contrary to what has long been believed. However, this trait appears to be quite complicated, being partly strain dependent and partly dependent on the host's immunity. Consistent definitions of persistence and more uniform methods for testing this phenomenon will benefit future research. The factors explaining the anticipated differences in pathogenic behavior appear to be more difficult to evaluate. Biofilm formation and the presence of various staphylococcal virulence factors do not seem to (directly) influence the effect of CNS on IMI but the available information is indirect or insufficient to draw consistent conclusions. Future studies on the effect, persistence, and virulence of the different CNS species associated with IMI would benefit from using larger and perhaps even shared strain collections and from adjusting study designs to a common framework, as the large variation currently existing therein is a major problem. Also within-species variation should be investigated.

Key words: coagulase-negative staphylococci (CNS), intramammary infection, effect, persistence, biofilm, virulence

CNS AND UDDER HEALTH

Mastitis and CNS

Mastitis is an inflammation of the mammary gland that commonly originates from IMI, most often caused by bacteria such as streptococci, coliforms, and staphylococci. The disease appears in 2 forms: either clinical, characterized by visible symptoms, sometimes general illness, and a long lasting negative effect on milk production, or subclinical, without visible symptoms but with an increase in SCC and suboptimal milk production. In the last decade, the CNS have become the microorganisms most frequently identified in cases of subclinical mastitis, arousing enhanced interest from mastitis researchers (Pyörälä and Taponen, 2009; De Vliegher et al., 2012).

Coagulase-negative staphylococci, along with other agents such as *Corynebacterium* spp., are commonly considered to be minor mastitis pathogens, as opposed to major pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus uberis*, and *Streptococcus agalactiae*. However, conflicting results on aspects such as the effect of CNS IMI on SCC and milk yield (**MY**), and the virulence potential and epidemiology of CNS found in milk, have caused confusion regarding the true importance of CNS for udder health. This confusion has led researchers to challenge the idea that CNS are a homogeneous bacterial group. Instead, the existence of species-specific differences has been predicted (Pyörälä and Taponen, 2009). Several recent studies, outlined

Received November 27, 2013.
Accepted May 12, 2014.
[1]Corresponding author: Sarne.Devliegher@UGent.be

below, have focused on the diversity among CNS species with respect to their role in ruminant udder health.

Main CNS Found in Ruminant Milk

A major drawback for looking beyond the border of CNS as a group has long been the lack of consistent identification and typing methods for isolates from nonhuman sources. Indeed, the routine phenotypic methods that were traditionally used to identify CNS species from milk were essentially developed for use with human isolates and were proven unreliable in case of (some) CNS species found in milk (Thorberg and Brändström, 2000; Taponen et al., 2006, 2008; Capurro et al., 2009; Sampimon et al., 2009b; Onni et al., 2010, 2012; Park et al., 2011a; Koop et al., 2012a). Recent studies have been able to overcome these difficulties as various validated molecular methods, including sequencing and fingerprinting methods, have become available (Piessens et al., 2010; Braem et al., 2011). If one now looks at studies presenting reliable species data, over 20 CNS species have so far been isolated from bovine milk (Taponen et al., 2007; Sampimon et al., 2009b; Park et al., 2011a; Persson Waller et al., 2011; Piessens et al., 2011; Supré et al., 2011; Mørk et al., 2012; Quirk et al., 2012). However, overall, just 5 species are commonly found: *Staphylococcus chromogenes*, being generally the most frequently detected species, *Staphylococcus simulans*, *Staphylococcus xylosus*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis*. Therefore, the rest of this review will focus mainly on these species, which will further be referred to as the 5 main species. Sometimes other CNS species from milk will be considered in addition to the 5 main species, such as *Staphylococcus caprae*, being one of the main species in milk from goats and sheep (Onni et al., 2010, 2012; Koop et al., 2012a), as will CNS species from other niches than milk, including humans or the bovine environment. Studies with species data resulting from (solely) phenotypic identification will occasionally be cited.

Determining Udder Health Status

Udder health can be expressed in various ways. First, the inflammatory response of the cow can be determined, which is most frequently done, both in practice and in research, through measuring the SCC. Other parameters such as *N*-acetyl-β-glucosaminidase (**NAGase**), milk amyloid A (**MAA**) level, serum amyloid A (**SAA**) level, and the level of proinflammatory cytokines interleukin or tumor necrosis factors have been considered (Simojoki et al., 2011; Kalmus et al., 2013). Second, the detection of visible signs, such as swelling, redness, and hardness of the udder, represents an obvious, macroscopic way to assess udder health. Interestingly, a significant positive association has recently been illustrated for the severity of the clinical signs with inflammatory markers in the milk (Kalmus et al., 2013). A third parameter, possibly the most appreciable for the farmer, is milk production, indirectly related to udder health and several other disorders of infectious or metabolic origin. These 3 aspects are all expressions of an inflammatory or other physical reaction of the host and, in this review, the effect of CNS will be discussed in relation to all 3. In addition, in some studies, only the presence, absence, or new occurrence of IMI is determined, without taking any reaction of the host in consideration. Also this approach to udder health will be discussed.

Persistence of IMI

Persistent IMI is viewed as a major issue related to staphylococcal mastitis. It refers to the occurrence of the same infectious agent in the milk throughout a certain period, such as the dry period or part of or even the entire lactation. However, assessing persistence of IMI especially requires consistent strain identification. Although persistence of an infectious agent can be suspected when the same species is detected in consecutive samples (Taponen et al., 2006; Supré et al., 2011; Koop et al., 2012b), reliable conclusions can only be made when appropriate typing methods have shown these samples to harbor identical strains (Rajala-Schultz et al., 2009). Concerning staphylococci, recent research has shown that when an udder quarter yields a series of samples positive for a certain *Staphylococcus* species over time, it is likely to be persistently infected (Mørk et al., 2012). Therefore, molecular studies only including species data can be argued to yield valuable insights on the persistence capacities of CNS, even though they are not conclusive.

Biofilm Formation and Other Putative Virulence Factors of Staphylococci

In general, 3 groups of virulence factors are involved in pathogenesis of staphylococcal infections: secreted proteins (e.g., superantigens, cytotoxins, and tissue-degrading enzymes), cell surface-bound proteins [e.g., microbial surface components recognizing adhesive matrix molecules (**MSCRAMM**)], and cell wall components (e.g., the polysaccharide capsule and lipoteichoic acid; Arvidson, 2006; Dedent et al., 2006; Novick, 2006; Atanasova et al., 2011). Besides their cytological origin, virulence factors can also be considered according to their role during pathogenesis [i.e., in adhesion to the host tissue (e.g., MSCRAMM, such as fibrinogen-binding protein and fibronectin-binding protein), in evasion