

## SUMMARY

### Gorik Braem

Prevalence of coagulase-negative staphylococci on the teat skin of cows in Flemish dairy herds

Mastitis is a complex intramammary infection that is affected by pathogenic bacteria and that may be due to multiple factors, including housing, hygiene, milking procedures, and feeding programs. Emphasis on higher milk production has caused an increased sensitivity of cows towards the development of mastitis. Although the implementation of mastitis control programs has successfully lowered the prevalence of contagious pathogens, the proportion of culture-positive milk samples still increases. This is mainly due to the presence of coagulase-negative staphylococci (CNS), which are often considered as minor pathogens. Currently, CNS are the most prevalent bacteria found in bovine milk samples and they are able to cause both clinical and subclinical mastitis. Nevertheless, there are also indications that CNS may have beneficial effects on udder health and milk quality.

The present dissertation focused on the elucidation of the CNS diversity present on the teat apices of lactating cows in Flemish dairy herds. The teat apex is of particular interest, as it is the last exterior body site before entrance to the teat canal and the subsequent intramammary tissue. To create a trustworthy method for the further elucidation of the CNS diversity, an extended reference framework for the classification and identification of different CNS isolates, originating from (sub)clinical milk samples and teat apex swabs, was constructed based on (GTG)<sub>5</sub>-PCR fingerprinting of genomic DNA, which achieved a typeability of 94.7 % and an accuracy of 94.3 % compared to identifications based on gene sequencing.

Sixty Friesian-Holstein dairy cows from six well-managed Flemish farms were included in an extensive field study. Both healthy and infected dairy cows were shown to have a wide bacterial diversity on the teat apices, which were not exclusively populated by CNS. The microbiota on the teat apices of dairy cows suffering from clinical mastitis in one quarter was mapped with a culture-independent method (V3-16S rRNA-PCR-DGGE). They belonged to four bacterial phyla, namely the Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, encompassing a total of 17 bacterial genera. Swab samples obtained from teat apices of clinically healthy lactating cows from two farms gathered during one month were analysed using a combination of (GTG)<sub>5</sub>-PCR fingerprinting, gene sequencing (16S rRNA and *rpoB* genes), and a novel PCR-DGGE technique based on the *tuf*-PCR amplicon. Despite disinfection of the teats approximately every 12 h, the teat apices remained contaminated by a wide species diversity between two milking rounds. Although in low numbers, a total of 20 different bacterial genera were

present, which belonged to three phyla (Actinobacteria, Firmicutes, and Proteobacteria). The genera present on the teat apices could be classified as commensal skin-associated bacteria, opportunistic pathogenic bacteria, and mastitis-causing pathogens. In particular, a wide diversity and occurrence of CNS was found. To elucidate the variability of the staphylococcal species diversity on the teat apices of dairy cows, all teats of ten cows per farm were sampled once a month throughout a year. Four CNS species were predominantly present on the teat apices, namely *Staphylococcus haemolyticus*, *Staphylococcus cohnii* subsp. *cohnii*, *Staphylococcus equorum*, and *Staphylococcus saprophyticus* subsp. *saprophyticus*. Teat apices were frequently colonized by these four species for several months. The remaining staphylococcal isolates consisted of 22 different species. The overall distribution of staphylococci showed fluctuations over time and was herd-dependent, with a possible relationship to the post-milking teat dipping in use.

Several CNS strains of the field study were screened for the prevalence of antibacterial activity. Out of a total of 254 CNS teat apex strains, belonging to 23 different species, 38 strains from twelve different species displayed inhibitory activity against an indicator strain (*Listeria innocua* LMG 13568). Out of these positive CNS strains, seven strains displayed inhibitory activity against at least one of the tested mastitis pathogens (strains of *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Staphylococcus aureus*). *Staphylococcus chromogenes* L217 excelled in its antibacterial activity, displaying a clear inhibitory effect against all tested mastitis pathogens and against 70 % of the 35 tested CNS teat apex strains, which belonged to 16 different species. Its antibacterial peptide was identified as a nukacin (nukacin L217). This is the first description of a bacteriocin produced by *S. chromogenes*.

The results obtained in this work contribute to a better understanding of the staphylococcal species diversity present on the teat apices of dairy cows. In particular, the presence of a wide diversity of CNS was revealed, all living in close association with each other and with a variety of other bacteria. In the light of a recent finding uncovering a wide diversity of antibiotic resistance markers in CNS, this mapping of the staphylococcal species diversity can give better insights into the potential risks associated with CNS in an environment under constant antimicrobial pressure. Additionally, the results indicate that some CNS offer the means to combat both mastitis-causing pathogens and other, potentially harmful CNS, through the production of bacteriocins.