VALIDATION OF tDNA-INTERGENIC SPACER PCR FOR SPECIES LEVEL IDENTIFICATION OF BOVINE COAGULASE-NEGATIVE STAPHYLOCOCCI

K. Supré[1*], S. De Vliegher[1], O. Sampimon[3], R. Zadoks[4], M. Vaneechoutte[5], M. Baele[2], A. de Kruif[1], F. Haesebrouck[2]

[1]Dept. Reproduction, Obstetrics and Herd Health and [2]Dept. of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, Belgium; [3]Animal Health Service, Deventer, The Netherlands; [4]Quality Milk Production Services, Cornell University, Ithaca, New York, USA; [5]Dept. Clinical Chemistry, Microbiology and Immunology, Faculty of Medicine, Ghent University, Belgium;[*]Corresponding author: karlien.supre@UGent.be

Introduction: In many European dairy farms that have adopted the 5- and 10-point mastitis prevention programs, the relative importance of coagulase-negative staphylococci (CNS) has increased. The increase in CNS prevalence relative to traditional major pathogens, combined with changes in limits for bulk milk somatic cell count penalties and the fact that CNS are causing clinical mastitis also, warrant reconsideration of their historical designation "minor pathogen". On the other hand, protective characteristics of CNS have been reported. The confusion can partly be explained by the lack of (accurate) species identification, which is a prerequisite for elucidating the importance of CNS. Current identification methods are largely phenotypic and based on human reference strains, but this may not be suitable for bovine CNS. Within the scope of our field study concerning CNS on dairy farms, we have been searching a molecular method that is cheap, rapid and easy to perform. At our laboratory, tDNA-intergenic spacer PCR (tDNA-PCR) is available but has never been validated for identification of bovine CNS. In this study, we have updated tDNA-PCR for identification of bovine CNS species by extending the current library of the technique, followed by comparing the results with sequencing of the *rpo*B-housekeeping gene.

Materials and methods: ***Isolates:*** For the extension of the tDNA-PCR library, 146 CNS isolates originating from bovine milk (94) and teat apices (52) were used (results not shown). To validate tDNA-PCR, 148 bovine CNS (100 and 48 from bovine milk and teat apices, respectively) were analysed and tDNA-PCR results were compared with gene sequencing. ***Techniques:*** tDNA-PCR[1,2,4] and sequencing of the *rpo*B-gene[3] were performed as described with small modifications. When there was no amplification with the *rpo*B-primers or less than 97% homology with reference strains was seen, additional *cpn*60-sequencing and if not sufficient *16S*-sequencing, was performed.

Results and discussion: When studying the impact of different CNS species on performances in dairy cattle, an accurate identification technique is required. Although no single test can offer fully reliable identification of bacterial species, gene sequencing is often seen as the gold standard. Unfortunately, the high cost and its labour intensiveness limit its use in large field studies for most routine laboratories. Phenotypic methods on the other hand are usually cheaper but lack accuracy. tDNA-intergenic spacer PCR is a rapid, low-cost and easy to perform technique that has a high reproducibility if capillary electrophoresis is available[2]. The results of this study show that tDNA-PCR could be a good alternative for gene sequencing. After updating the current library, the overall agreement (isolates from milk and teat apices) between tDNA-PCR and gene sequencing was 97.5%. When focussing on milk samples and teat apices separately, 96.6 and 100% of the identifications agreed in both tests, respectively. Overall, a high number of CNS-isolates could not be identified with the gold standard (gene sequencing: *rpo*B, *cpn*60, *16S*), especially isolates originating from teat apices (20.3%). Possible explanations could be the presence of undefined species on teat apices, or strain differences between isolates from different origins. Still, availability of a complete reference database is a prerequisite and could be the bottleneck. Additional sequencing of the *tuf*-gene might give a definite answer (in progress). To conclude, tDNA-PCR will be a useful tool for our field study aiming at elucidating the relevance and epidemiology of CNS in dairy cattle, and its use in other laboratories should to be promoted.

Acknowledgements: This research is funded by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen, grant n° 61459). The authors acknowledge the Dutch Udder Health Centre, Deventer, The Netherlands, for financially supporting part of the analyses.

References: can be obtained from the corresponding author.