Abstract of my research work

*** Name:** Yasser Saadeldien Ibrahim Mahmmod

*** Public e-mails;** yasser@sund.ku.dk ; yasserpcr@gmail.com

*** Home institute address:**

Department of Animal Medicine,
Faculty of Veterinary Medicine,
Zagazig University,
44511- Zagazig,
Egypt

*** Host institute address:**

Department of Veterinary and Animal sciences,
Faculty of Health and Medical Sciences,
University of Copenhagen,
1872-Frederiskberg,
Denmark

*** Main Project title:**

Udder Health and Control of Contagious Mastitis in Dairy Cows

Titles and abstracts of subprojects and/or papers include:

1- DNA carryover in milk samples from routine milk recording used for PCR-based diagnosis of bovine Staphylococcus aureus mastitis. Published as a full original research article in Journal of Dairy Science. 2017 Jul;100(7):5709-5716. doi: 10.3168/jds.2016-12330.

Yasser Mahmmod, Ilka Klaas, Carsten Enevoldsen

Abstract

Real-time PCR techniques are increasingly used to detect udder pathogens from milk samples collected non-aseptically at routine milk recording. The objectives of this study were (1) to estimate the statistical associations between cycle threshold (Ct) values for Staphylococcus aureus in non-aseptically collected composite samples taken at routine milk recording from cows milked consecutively with the same milking unit and milk meter; and (2) to formulate practical and plausible guidelines for understanding the diagnostic implications of PCR testing for Staph. aureus intramammary infection at routine milk recording. The study included 4 herds with conventional milking parlors and repeatedly low Ct-values for Staph. aureus (representing a high DNA load) in bulk tank milk. Composite milk samples were collected from all cows at all milking units during routine milk recording using the Tru-Test electronic milk meter (Tru-Test Group, Auckland, New Zealand) and analyzed using the PathoProof PCR (Thermo Fisher Scientific, Vantaa, Finland) assay. Milking clock times were retrieved at each milk meter to establish the milking order of the cows at each unit. A multinomial logistic regression was applied to estimate the association between Ct-values from cows milked consecutively with the same milking unit and milk meter. The following groups were selected based on Ct-values: (1) 0-31.3, (2) 31.4-33.9, (3) 34.0-37, (4) 37.1-39.9, and (5) 40 (negative result). The association between groups from cows milked consecutively with the same milking unit and milk meter was statistically significant. Approximately 60% of cows were in Ct group 5 if the antecedent cow was also in Ct group 5, but only 20% of cows were in Ct group 5 if the antecedent cow was in Ct group 1. The probability of cows being in Ct group 1 was not markedly influenced

by the group of the antecedent cow. Statistical relationships in the intermediate range gave a plausible indication of a dose-response relationship. Carryover of bacterial DNA via the milking unit and milkmeter is very likely to affect PCR results for Staph. aureus. Therefore, information about milking order must be considered in mastitis control efforts. We suggest a practical interpretation of PCR results: cows with a Ct-value <32 can be labeled "very likely to be infected with Staph. aureus," but cows with Ct-values of >37 and 32-37 can be labeled "very likely to be negative for Staph. aureus" and "uncertain Staph. aureus status," respectively.

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2- Distribution of Coagulase-Negative Staphylococci Species on Teat Skin and in Milk Samples from Dairy Cows in Automatic Milking Systems. Published as an oral presentation at Second seminar on coagulase-negative staphylococci, 18-19 May, Ghent, Belgium 2017.

Yasser Mahmmod, Line Svennesen, Karl Pedersen, Ilka Klaas

Abstract

Coagulase-negative staphylococci (CNS) frequently colonize teat skin and are one of the most common findings cultured from milk samples of cows with subclinical intramammary infections (IMI). Several species are related to IMI, but knowledge about the epidemiology of CNS species is limited. Cows in automatic milking systems (AMS) may have increased risk for teat colonization and IMI because more than 60 cows are milked several times daily with the same milking unit. The objectives of this study are (1) to investigate patterns of CNS species in milk samples and teat skin swabs in nine AMS herds and (2) to identify the predisposing cow level risk factors for specific CNS IMI and teat colonization.

In each herd, 30- 40 cows with somatic cell counts > 200,000 cells/ml in the previous milk recording are randomly selected. Cows treated for mastitis during the time between milk recording and sampling are excluded. Teat skin swabs and aseptic quarter foremilk samples are taken from all quarters of all selected cows. Teat skin swabs are collected using the modified wet-dry method. Briefly, sterile swabs are rotated 360° around the teat canal orifice, first a wet swab immersed in ¼ Ringer's solution, then a dry swab. Immediately after sampling, the tips of both swabs are transferred into one tube with 2 ml of ¼ Ringer's solution. Samples are transported on ice for culturing in the laboratory. After vortexing, 0.01

mL of each quarter milk sample and 0.1 mL of each quarter teat swab are streaked simultaneously on Staphylococcus selective medium (SA Select) and calf blood agar. Colonies from quarters suspect of having CNS in milk and/or teat skin samples (cut-off five CFU) are subjected for MALDI-TOF assay for species identification. Only isolates from the right hind and left front quarters are analyzed by MALDI-TOF assay in this study. To date, preliminary results of milk and teat skin samples from 130 quarters (65 cows) are available. CNS species were identified in 86 quarters out of the total number (130), representing 69 teat skin swabs and 17 milk samples. Out of the CNS positive quarters (n= 86), 12 quarters (11 teat skin swabs and one milk sample) were harboring more than one type of the CNS species. *Staphylococcus epidermidis* and *Staphylococcus equorum* were the most frequently isolated CNS species from milk samples (7/17) and (5/17), respectively. *Staphylococcus equorum*, *Staphylococcus haemolyticus* and *Staphylococcus xylosus* were the most frequently isolated CNS species from teat skin swabs (56/69), (9/69), and (6/69), respectively. *Staphylococcus cohnii* (n= 2), *Staphylococcus saprophyticus* (n= 1) and *Staphylococcus hominis* (n= 1) were identified only in teat skin swabs while *Staphylococcus simulans* (n= 1) was only identified in milk samples. *Staphylococcus chromogenes* was detected in both milk (n= 2) and teat skin (n= 1) samples. Data collection will be finished in April 2017. The final results will give new insights into herd specific CNS species patterns and the microbial ecology and epidemiology of common CNS species from different habitats – teat skin and milk. We hope that our findings improve the udder health, milk quality and control of mastitis caused by different CNS species in dairy herds with AMS.

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3- Distribution of non-aureus staphylococci from teat and milk samples in dairy herds with automatic milking system and their interaction with the *agr* quorum sensing system of *Staphylococcus aureus*. Full Manuscript submitted to Veterinary Microbiology

Yasser Mahmmod, Line Svennesen, Hanne Ingmar, Karl Pedersen, Ilka Klaas

Abstract

Information about the epidemiological and ecological characteristics of coagulase negative staphylococci (CNS) in dairy herds with automatic milking systems (AMS) is sparse hence, identifying these characteristics is crucial for improving the udder health management in

AMS. Furthermore, the role of CNS on the risk of acquisition of intramammary infections (IMI) with *S. aureus* is vague and yielded long-time debate. The objectives of this study were to (1) investigate the distribution patterns of CNS species on quarter level from aseptic milk and teat skin in dairy herds with AMS, and (2) examine if the isolated CNS influence the expression of *S. aureus* virulence factors controlled by the *agr* quorum sensing system. The study was conducted on 8 dairy herds were selected for having AMS and ≥ 3 milking robots. In each herd, 30-40 cows were randomly selected and included based on the criteria of having SCC ≥ 200,000 cells/mL at the last milk recording and not subjected for antibiotic therapy in the four weeks prior to sample collection. Teat skin swabs and aseptic quarter foremilk samples were taken from right hind and left front quarters of cows with odd number. Teat skin swabs were collected using the modified wet-dry method and milk samples were taken aseptically for bacterial culture. Colonies from quarters with suspicion of having CNS in milk and/or teat skin samples were subjected to MALDI-TOF assay for species identification. To investigate the interaction between *S. aureus* and CNS, 81 isolates CNS were subjected qualitative beta-galactosidase reporter plate assay.

In total, 16 different CNS species were identified from 284 quarters (= 142 cows), where 15 species were identified from teat skin and 10 species identified from milk. Out of 518 isolates, 373 (72%) isolates were successfully identified. Based on sample type, 105 CNS isolates were identified from milk and 268 isolates were identified from teat skin. The frequency of mixed infections of CNS species per quarter (n=70) in teat skin was higher than milk (n=11). The most prevalent CNS species identified from milk were *S. epidermidis* (52/105), *S. haemolyticus* (17/105), and *S. chromogenes* (10/105), while the most identified CNS species from teat skin were *S. equorum* (114/268), *S. haemolyticus* (42/268), and *S. cohnii* (40/268). There was strong significant association (*P value* <0.0001) between the frequencies of CNS species isolated from milk including *S.arlettae, S.chromogenes, S.cohnii, S.epidermidis, S.equorum, S.haemolyticus, S.hominis*, and *S.xylosus*, and those isolated from teat skin. There was no significant association between the frequency of CNS species isolated from left front and right behind quarters, while there was strong significant association between the frequencies of CNS species among the 8 dairy herds (*P value* <0.0001). Herd-to-herd differences in distribution of CNS species were observed in both milk and the teat skin, suggesting that herd management are involved in the establishment of particular CNS species. Using reporter gene fusions monitoring transcriptional activity of key virulence factors and regulators, we found that out of 81 CNS isolates, supernatants of

58 (71.6%), 55 (67.9%), and 49 (60.5%), reduced expression of *hla* encoding a-hemolysin and -RNAIII, the key effector molecule or *agr,* while increasing expression of *spa* encoding Protein A, respectively. Our finding indicate that the majority of CNS strains isolated interfere with the *agr* quorum sensing system of *S. aureus*. The CNS species variably influenced the activity of virulence factor expression in *S. aureus* with the effects of *S. chromogenes, S. equorum* and *S. xylosus* correlating with *agr* repression while *S. sciuri*, *S. cohnii and S. capitis* did not influence expression of *agr*-controlled genes.

The findings of this study demonstrate extensive diversity of CNS species and their relation with *S. aureus* IMI and/or colonizing teat skin of dairy cows. The knowledge of how CNS influence S. aureus virulence factors expression may ultimately help in controlling *S. aureus* IMI. We hope that our findings improve the udder health, milk quality and mastitis control in dairy herds with AMS.