

NOTES ON SOMATIC CELLS COUNT IN DAIRY MASTITIS DETECTION

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Summary

The study was carried out during the fall season, in one dairy farm, on somatic cells count (SCC) collected from cows on the same day as milk samples for microbiology testing of mastitis. Transversal and retrospective analyses of SCC were carried on in order to observe a possible association between detection by SCC, California Mastitis Test positive (CMT+), milk quality indicators and microbiological mastitis. From a BIOAMR data basis, 60 cows subclinical and microbiological diagnosed with mastitis were sampled - in 70% of cases was identified *Staphylococcus aureus*. The SCC were retrospectively correlated with previous' milk urea nitrogen or MUN ($r = +0.655$, at $p = 0.001$), SCCs' trend ($r = + 0.815$, at $p = 0.000$) and transversal correlated with protein ($r = + 0.342$ at $p = 0.031$), lactose ($r = + 0.357$ at $p = 0.024$) and density ($r = + 0.320$ at $p = 0.044$). The fifth classes of SCC was retrospectively associated with fat ($p = 0.040$), MUN ($p = 0.022$), and transversally associated with SCC trend ($p = 0.000$), freezing point ($p = 0.060$), number of glands CMT + ($p = 0.006$), left front tits CMT + ($p = 0.027$), and rear right tits CMT+ ($p = 0.060$). The SCC was easily associated in retrospective or transversal approach of milk quality indicators to CMT, but the study cannot found the association between specific germs (including MRSA) of mastitis and SCC. On such condition, further study has to be developed in order to improve the detection more specifically in the presence of pathogen bacteria.

Keywords: somatic cells count, milk constituents, mastitis detection

In clinical mastitis, signs such as abnormal milk (changes in color, presence of clots, flakes), abnormal mammary gland (changes in tissue color, swelling) and changes in animal status (14) such body temperature (10), appetite, and hydration level, are easily visible. In subclinical mastitis the majority of previous signs are not detectable (1, 2). In both cases the somatic cells count can easily be monitored (12).

Somatic cell count (SCC) is the most common method for the detection of mastitis. If the detection is followed by diagnosing the bacterial agent, the results can help to determine treatment and prevention strategies on the farm, which in turn can help to reduce incidence and prevalence (1).

The aim of the study is a transversal and retrospective study of association of SCC with milk constituents in cases of animals *a posteoriori* diagnosed with pathogenic germs of mastitis.

Materials and methods

Farms and animals sampling: 20 partner farms of Extension unit from four counties of West Romania were stratified sampled (5 farms for each of the counties Arad, Bihor, Timis and Hunedoara) in a screening for dairy mastitis infection. All farms were included and follow the Official Control of Milk Production managed by regional Breed associations (7-9, 11). From the sampled farms, 60 cases clinically and microbiologically diagnosed with mastitis of the infected cows' quarts of udders were included in the retrospective study the infected cows' quarts of udder.



Fig. 1. CMT & collecting the milk samples for primary analysis at the farms level

Detection of mastitis with California Mastitis Test and milk sampling for positive CMT cows at the milking parlor site (left). Animal Production Laboratory (right). Analyzing the milk CMT positive sample for milk constituents and content (Funke Gerber Lactostar Dairy Analyser) and somatic cells count (DeLaval cell counter DCC).

Source: UEX Media, 2019. The student team of Bioeconomic approach to antimicrobial agents - use and resistance project during the farm visits activities.

Data collection and processing: the Californian Mastitis Test (CMT) and milk samples have been taken and primarily analyzed (Figure no. 1) on the farm for all dairy cows. Only positive sample to CMT were collected for analyzing the chemical milk constituents (*Funke Gerber Lactostar Dairy Analyser*) and SCC analysis (*DeLaval cell counter DCC*). The SNF (fat free dry matter), protein, fat, lactose and minerals with maximum ± 0.04 % reproducibility were measured, and freezing point and density were calculated.

Microbiology analysis of samples collected by COPAN's *ESwab™* system was effectuated for all CTM positive quarters. Each infected quarter was considered an individual sample. The germs were isolated by classical microbiological exam. Commercial culture-based tests are available for diagnose of mastitis such us: CHROMagar Mastitis (CHROMagar, France), Hardy Diagnostics Mastitis Triplate (Hardy Diagnostics, USA), Minnesota Easy Culture System II Triplate (University of Minnesota, USA), and Vétorapid (Vetoquinol, the Netherlands) (4). The types of germs and antimicrobial resistance (AMR) were analyzed by *Walk Away System* using *MicroScan® Dried Panels*.

Statistical Analysis: SPSS® *Statistics* software for *Spearman's* correlation, χ^2 test, *Mann-Whitney U test* were used in order to do the analyses of association, frequency and differences between SCC trend and several groups and variables of the study. The hypothesis was accepted at the lever of value $\alpha = 0.05$.

Results and discussions

Milk from cows with mastitis cannot be used for consumption because it has altered chemical composition and organoleptic proprieties. In juridical prospect, the maximum level of CSS has to be maximum 400.00×10^3 cell/ml of milk. In the collected sample, the average value of SCC was more than three time mode - $1,426.18 \pm 244.99 \times 10^3$ cells/ml of milk. So, by the number of somatic cells it is clear that milk cannot be used for human consumption but, by diluted in the culling tank with normal milk, the average of SCC in milk tank decreases under legal limit. However, some studies have proven an increased prevalence of resistant bacteria from dairy animals undergoing antibiotic treatment (3). In practice using non-salable milk from cows under antibiotics treatment to feed the calves will increase the risk of increasing fecal shedding of AMR bacteria (13).

The SCC were retrospectively correlated with previous' milk urea nitrogen or MUN ($r = +0.655$, at $p = 0.001$), SCCs' trend ($r = + 0.815$, at $p = 0.000$) and transversal correlated with protein ($r = + 0.342$ at $p = 0.031$), lactose ($r = + 0.357$ at $p = 0.024$) and density ($r = + 0.320$ at $p = 0.044$). The fifth classes of SCC was retrospectively associated with fat ($p = 0.040$), MUN ($p = 0.022$), and transversally associated with SCC trend ($p = 0.000$), freezing point ($p = 0.060$), number of glands CMT + ($\chi^2 = 33.90$ at $p = 0.006$), left front tits CMT + ($\chi^2 = 10.94$ at $p = 0.027$), rear right tits CMT+ ($\chi^2 = 9.03$ at $p = 0.060$).

By transversal analysis, the positive bacterial samples (which were positive to bacteria) SCC was associated with sensibility and AMR to Clindamycin ($\chi^2 = 22.97$ at $p = 0.003$), Linezolid ($\chi^2 = 11.66$ at $p = 0.020$), Netilmicin ($\chi^2 = 17.94$ at $p = 0.001$), Synercid ($\chi^2 = 26.30$ at $p = 0.001$) and Vancomycin ($\chi^2 = 20.77$ at $p = 0.008$). In retrospective analysis, the trend of SCC was associated with sensibility and AMR to Clarithromycin ($\chi^2 = 6.98$ at $p = 0.030$), Clindamycin ($\chi^2 = 8.18$ at $p = 0.017$), Erythromycin ($\chi^2 = 8.80$ at $p = 0.012$), Linezolid ($\chi^2 = 6.98$ at $p = 0.033$),

Netilmicin ($\chi^2 = 4.06$ at $p = 0.044$), Synercid ($\chi^2 = 13.62$ at $p = 0.001$) and Vancomycin ($\chi^2 = 8.88$ at $p = 0.012$). In this case, the trend was associated with AMR detection for more times. In comparison with the transversal study, the retrospective index works much better.

The SCC was easily associated in retrospective or transversal approach (8) of milk quality indicators to CMT, but the present study did not find the association between specific germs (including MRSA) of mastitis and SCC. May be analyzing other biomarkers, such as released enzymes reflecting tissue destruction (*Lysosomal N-acetyl- β -d-glucosaminidase - NAGase or Lactate dehydrogenase - LDH or LD*) will improve the association between detection and diagnosis of mammary gland inflammation (5, 6).

Conclusions

In terms of results provided by SCC detection, the dynamic or retrospective analysis works better than the transversal approach.

The study cannot found the association between specific germs (including MRSA) of mastitis and somatic cell count (SCC).

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