Retrospective analysis on somatic cells count trend in *Staphylococcus aureus* cows' mastitis

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Abstract

The study was carried on detection of mastitis, using increasing respectively decreasing trend of somatic cells count (SCC) of two successive samplings. The analysis was performed to identify the associations between SCC trend with retrospective and transversal milk quality indicators, and mastitis. From a BIOAMR database, 28 cows with ***a posteriori*** *diagnosed mastitis with Staphylococcus aureus (4/28 cases were methicillin-resistant Staphylococcus aureus – MRSA) were sampled. The trend of SCC was Spearman's rho correlated with previous lactose (r_s = +0.785, p=0.03), pH (r_s = +0.662 at p=0.019), and current SCC measurements (r_s = +0.781, at p=0.000). Increasing trend of SCC was retrospectively associated with lactose content (Z = -2.152 at p = 0.031), pH (Z = -2.152 at p = 0.031) SCC at first measurement (Z = -1.764 at p = 0.078) and currently associated with SCC (Z = -3.316 at p = 0.001), fat content (Z = -1.88 at p = 0.060) and fat/protein ratio (Z = -1.717 at p = 0.086). The 28 samples of the study did not revealed strong association between SCC trend and type of Staphylococcus aureus (p = 0.186 by Mann-Whitney test), even if MRSA had a higher increasing trend of SCC in comparison whit S. aureus (non-MRSA) infections (1403.5 vs. 288.2 thousands somatic cells). By preliminary results the trend of somatic cells could be an indicator in detection of mastitis but more case studies are necessary.*

Keywords: *Staphylococcus aureus, somatic cell count, cow mastitis and AMR.*

Introduction

Staphylococcus aureus is one of the main contagious pathogens responsible for the intra-mammary infection in dairy cattle and mastitis is one of the most economically important health traits for milk production *(6).* Detection of mastitis is generally based upon a number of indicators of mammary gland inflammation. The detection of the inflammation is based upon the response of the body to the mammary gland infection. The aim of the study is detection - diagnosed remarks in a retrospective study of the trend of somatic cells count (SCC) as two successive measurements in association with changes in milk, in order to improve future detection and treatment patterns.

Materials and methods

Farms and animals sampling: 15 partner farms of Extension unit in three counties of West Romania were stratified sampled (5 farms for each of the counties Arad, Bihor and Timiș) in a screening for dairy mastitis infection. All farms are included in the Official Control of Milk Production managed by Breed associations *(8,9,10,11)* - the last SCC value preview farm visit was considered. The SCC trend was calculated for in 11.2 ± 1.15 days' distance between two successive measurements. From overall *Bioeconomic approach to antimicrobial agents - use and resistance (acronym BIOAMR)* database 28 cases diagnosed with *Staphylococcus aureus* mastitis were

studied. In the study, from the sampled BIOAMR database, 28 cows with *a posteriori* diagnosed mastitis with *Staphylococcus aureus* (4/28 cases were methicillin-resistant Staphylococcus aureus – MRSA) were used.

Data collection and processing: the Californian Mastitis Test (CMT) and milk samples have been taken and primary analyzed (figure 1) on the farm for all dairy cows. Only positive sample to CMT were collected for chemical milk constituents (*Funke Gerber Lactostar Dairy Analyser*) and SCC analysis (*DeLaval cell counter DCC)*. Such chemical milk analysis device features fully automatic cleaning and rinsing system and zero point calibration for fast and accurate testing. The SNF (fat free dry matter), protein, fat, lactose and minerals with reproductibility maximum ± 0.04 % were measured and freezing point and density was calculated.

Microbiology analysis. The microbiological samples were collected by *COPAN's ESwab*^{TM} liquid collection and transport system form all positive quarters. Each infected quarter was considered an individual sample and only the most affected quarter (higher number of SCC) of one animal was included in the study. The germs were isolated and later, by microbiological exam, other 28 cases were used for the retrospective study. The typifying and antibiotic resistance was done by *Walk Away System* using *MicroScan® Dried Panels.*

Figure no. 1: Collecting and primary analysis of the samples at the farms level
CMT screening of the dairy cows in the milking parlor, in order to detect mastitis (*left*). Analyzing the milk positive sample for milk constituents *(Funke Gerber Lactostar Dairy Analyser)* and content, and somatic cells count (*DeLaval cell counter DCC*) – in the Animal Production Laboratory (*right*). *Source: UEX Media, 2019*

Statistical Analysis

The trend of SCC was considered positive or negative, depending on the value difference between the SCC measurement on the day of the farm visit, and the preview measurement, usually from results of Official Control of Milk Production. *SPSS® Statistics* software for *Spearman's* correlation, *Mann-Whitney U test* and nonparametric tests were used in order to do the analyses of association, frequency and differences between SCC trend and several groups and variables of the study. Significance value was accepted to be $\alpha = 0.05$.

Results and disscution

Milk somatic cells (considered as a Somatic Cells Count) are a mix of milk-producing cells and immune cells. Various factors and management practices modulate SCC and hematological parameters in a dairy herd: udder inflammation, parity, stage of lactation, unhygienic and incomplete milking, hot-humid climate, change in housing and feed or distress increase the SCC. Otherwise, healthy udder, antioxidants, hygienic milking, proper cow therapy, selection against mastitis, lower the *SCC (3,7,12,13,14).*

Staphylococcus aureus classified as a contagious pathogen, which can efficiently adapt to the environment of the mammary gland and spread cow to cow during milking, was considered in association with SCC. Several changes occur in blood *(4),* tissues and in milk *(5),* as a reaction to infection, including infiltration of leukocytes (measured by somatic cells content - SCC) and increased vascular permeability, resulting alterations in the chemistry of the milk resulting from hydrolysis of milk proteins by hydrolytic enzymes and oxidative substances released from phagocytes, alterations in milk pH and ionic solutes, and ingestion of milk components by phagocytes.

The trend of SCC was positive *Spearman's rho* correlated with previous lactose (r_s = +0.785, p=0.03), density (r_s = +0.662 at p=0.019), and with the SCC measurements on the day of the farm visit (r_s = +0.781, at p=0.000). Increasing trend of SCC was retrospectively associated with lactose content (Z = -2.152 at p = 0.031), density (Z = -2.152 at p = 0.031) SCC at first measurement (Z = -1.764 at p = 0.078) and currently associated with SCC at farm visit time (Z = -3.316 at p = 0.001), fat content (Z = -1.88 at p = 0.060) and fat/protein ratio (Z = -1.717 at p = 0.086). The 28 samples of the study did not reveal strong association between SCC trend and type of *Staphylococcus aureus* (p = 0.186 by *Mann-Whitney* test), even if MRSA had a higher increasing trend of SCC in comparison whit *S. aureus* (non-MRSA) infections (1403.5 vs. 288.2 thousands somatic cells).

The lower SCC trend can be associated with the capacity of the body to react to infection; the higher trend can be associated with infection. In fact, the percentage of noninfectious and infectious cells from SCC is, and can be, established. The percentage of leukocytes in SCC in milk is different in healthy cows vs. infected. *Alhussien et. al. 2016 (1,2)* reported 19% vs. 75% neutrophils (diameter 12-15 μm, nucleus is multi-lobed with bridges), 66% vs. 17% macrophages (diameter 20-30 μm, the largest cell type in milk) and lymphocytes 15 vs. 8% (diameter 9-16 μm, deeply stained round nucleus with low cytoplasm).

By preliminary results and corroboration with other study *(5),* the trend of somatic cells was proven to possibly be an indicator in detection of mastitis, but more studies are necessary.

Conclusions

- The higher trend of somatic cells count was associated with persistency of mastitis caused by '*S. aureus*
- The trend of somatic cells count was retroactively and positive correlated with milk lactose content and density.
- Trend of somatic cells count is associated with SCC, fat content and fat/protein ratio, on the day of the on-farm visit.

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