



## IMPLEMENTATION OF STRATEGIES FOR MASTITIS CONTROL IN DAIRY HERDS IN MACEDONIA: A CASE REPORT

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### ABSTRACT

Mastitis is probably the most common and costly disease in modern dairy cow husbandry. The aim of the present paper was to report the results concerning udder health after implementation of a specific strategy using both field and laboratory methods. During the period June 2010-December 2011 a total of 674 dairy cows from four dairy farms were included in the investigation. Clinical mastitis was diagnosed by detection of signs of inflammation in the udder, while subclinical mastitis was diagnosed at the animal level by an increased Somatic Cell Count (SCC) using laboratory tests, and subsequently confirmed at quarter level by California Mastitis Test (CMT). Microbiological analysis of the milk samples was carried out by standard procedures using Gram staining, biochemical tests and automated identification system.

The distribution of somatic cell counts on cow level (n=674) was: 305 (45.3%) with SCC less than 100,000 SCC/mL, 236 (35.0%) 100,001 - 350,000 SCC/mL, and 133 (18.7%) with more than 350,000 SCC/mL. From a total of 1684 quarters tested by CMT, 644 quarters (38.2%) were positive and 1040 quarters (61.8%) were negative. In 60 out of 101 quarters that had a positive CMT result and no current treatment and that were sampled for bacteriology, bacteria could be isolated. Main bacteria identified, were coagulase - negative staphylococci (40.0%), *Streptococcus agalactiae* was present in 25.0%, *Escherichia coli* in 16.6%, *Proteus* spp. in 11.7% and *Staphylococcus aureus* in 6.7% of the bacteriologically positive samples. After introducing specific mastitis-control measures, focusing on milking hygiene, dry-off treatment, and antibiotic treatment of both clinical and sub-clinical mastitis cases, the prevalence of subclinical mastitis was reduced from 38.2 to 10.8%, while the incidence of clinical mastitis decreased from 21.0% to 4.9%.

In conclusion, the implementation of a standard mastitis control plan based on a regular assessment of the somatic cell count can reduce the prevalence of clinical and sub-clinical mastitis in dairy herds.

**Key words:** mastitis, CMT, dairy cows, SCC

### INTRODUCTION

Mastitis is probably the most common and costly disease in dairy cows (6, 15) because it affects a high proportion of animals, significantly decreases production and alters the milk composition. Several species of bacteria are able to invade the mammary gland, multiply there, and produce harmful

substances which may result in an inflammatory response (11). Clinically, this inflammatory response is visualized by swelling, redness, alterations in milk composition and decreased milk production of the infected quarter. Subclinical mastitis is manifested by elevated somatic cells without clinical signs and an obvious decreased milk production.

Significant advances in the control of mastitis in dairy herds have been achieved over the past 40 years (2, 5). Since recently, coagulase-negative staphylococci (CNS) are regarded as major contributors to bovine intra-mammary infections (mastitis) in modern dairy herds. They have been

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isolated from milk samples collected from cows with clinical and subclinical mastitis in several countries (1, 4). For some reason, heifers and primiparous cows seem to be more susceptible to CNS mastitis. On the other hand, these bacteria have also been shown to be protective against infections caused by major pathogens (12). Many CNS species can also be isolated from cows' hair coat, udder skin and teat canals, and are therefore often considered to be opportunistic skin organisms rather than real mastitis pathogens (14). Counting the somatic cells present in a milk sample is a common method for estimating the presence of mastitis and is often included in testing the milk quality at herd level. It is generally accepted that the average number of somatic cells from healthy cows is below 250,000/ml. (3). The "standard mastitis control plan", as proposed by Neave et al. (1969), and that comprises 5-points: appropriate treatment of clinical mastitis, culling of chronically infected cows, post-milking teat disinfection, correct maintenance and use of the milking equipment, and application of blanket dry cow therapy, was used with considerable success in reducing the prevalence of subclinical and clinical mastitis in many dairy herds (2). Vasil, 2007 (13) reported that proper milking hygiene leads to minimization of the teat-end contamination with pathogenic bacteria between milkings as well as during the milking process.

The aim of the present case report was to illustrate the results that were received after implementation of a strategy using field and laboratory methods to reduce udder health problems in some dairy herds in Macedonia.

## MATERIALS AND METHODS

During the period June 2010 to December 2011, a total of 674 dairy cows from four dairy farms in the Republic of Macedonia (Farms A, B, C and D) were included. In farm A, cows were housed in free stalls with cubicles, and milked twice daily in a herring-bone milking parlour of 16 cows per row. Cows were divided into several groups depending on the stage of lactation, the daily milk production level, and the health and pregnancy status. In the other 3 farms (B, C, and D) cows were housed in tied-stalls on deep straw bedding and milked twice daily.

Detection of subclinical mastitis at quarter level was done by the California Mastitis Test (CMT), consisting of a test plate with four separated wells to sample each quarter individually and California Mastitis reagent which is essentially a detergent. The detergent reacts with the cells present in the milk and forms a gel. Depending on the viscosity of the formed gel, the test was evaluated as negative or positive. A positive test (gel with high viscosity), usually indicates a SSC of higher than 400,000 cells/mL, indicating the presence of a subclinical infection in the tested quarter.

The somatic cell counts at cow level were evaluated by Fossomatic 5000 (Foss Electric, Denmark). The principle is as follows: the cells present in the sample are stained with a fluorescent dye, ethidium bromide, which forms chemical complexes with the DNA of the cell nuclei. When the cells are exposed to light of a specific wavelength, they emit fluorescent (red or orange) light. The light of each cell is detected and the number of total emitted light impulses (signals) is measured. The number of signals is displayed on a monitor.

Bacteriologic cultures were performed after aseptic collection of milk samples (n=101). Milk samples for culture were taken from each positive CMT quarter/s that was not treated with antimicrobials in the last 14 days. The culture was carried out using a standardised method. Briefly, 10 µL milk was inoculated on a Blood agar plate (Biorad, USA). The plates were incubated at 37°C for 24-48 hours in anaerobic conditions. After the incubation, suspected colonies were inoculated on Nutrient agar plates (Fluka, Switzerland) for 24h at 37±2°C in aerobic conditions. Each colony was assessed using standardised operating procedures for Gram staining, and further biochemical tests (oxidase and catalase activity) were applied to further identify the isolated bacteria. The identification of the pathogen was confirmed using the automatic biochemical identifying system Vitek 2 Compact (Biomérieux, France) using Gram-Positive and Gram-Negative cards (Biomérieux).

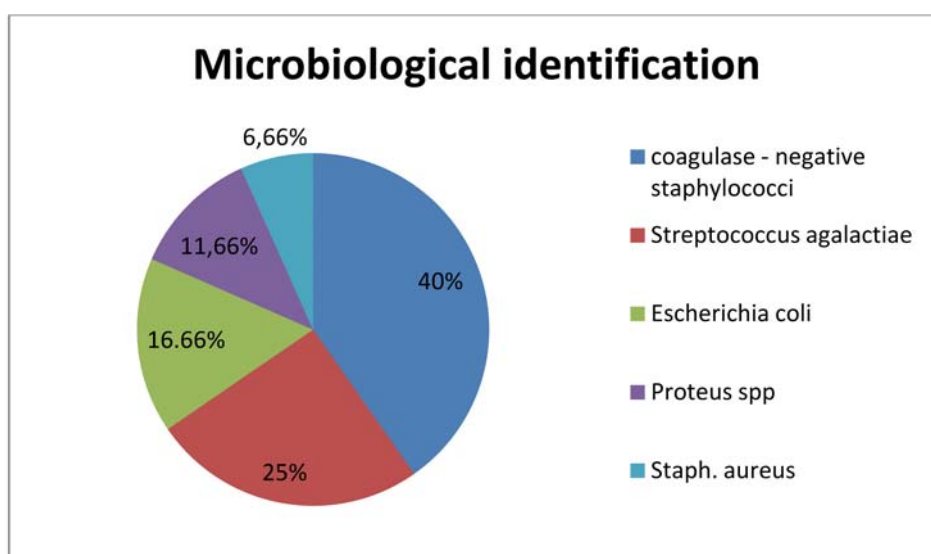
## RESULTS AND DISCUSSION

The distribution of the cow-level somatic cell count (n=674), was 305 (45.3%) with SCC less than 100,000 scc/mL, 236 (35.0%) 100,001 - 350,000 scc/

mL, and 133 (18.7%) with more than 350,000sc/mL. From a total of 1684 quarters tested by CMT, 644 quarters (38.2%) were positive and 1040 quarters (61.8%) were negative.

From a total of 101 milk samples taken for bacteriology, 60 samples (59.41%) were positive. The main aetiological agents of intra-mammary infections were coagulase - negative staphylococci (CNS) with 40%, (*Staphylococcus xylosum*, *Staph. epidermidis*, *Staph. saprophyticus* and *Staph. hominis*). *Streptococcus agalactiae* was isolated in 25%, *Escherichia coli* in 16.66%, *Proteus* spp. in 11.66% and *Staphylococcus aureus* in 6.66% of the samples.

Koivula et al., 2007(7) reported that CNS is generally associated with subclinical mastitis, which was confirmed in the present investigation. In such cows, CNS cause an increase in the number of somatic cells by 2 to 3 in comparison to uninfected quarters, which can be detected by CMT (9). The aetiological agents of mastitis on the farms participating in the present report were not dissimilar to those reported elsewhere (10). Therefore, it was expected that the same control measures that had previously been reported to lower the prevalence of sub-clinical mastitis and the incidence of clinical mastitis would be efficacious.



**Figure 1.** Results of microbiological identification in the quarters of the intra-mammary infections.

After the preliminary assessment and collection of the required samples, standard mastitis-control measures were introduced (dry cow therapy, hygiene program and prompt treatment of clinical cases). From the beginning of the observation, every cow entering the dry period was treated with a long acting antibiotic preparation immediately after the last milking. The hygienic program applied during milking consisted of pre-dipping, drying and cleaning of the teats and examination of the pre-milking fore-strips, while after milking the teats were dipped in an iodine-based teat disinfectant. The treatment of clinical mastitis cases was done using intra-mammary antimicrobials (amoxicillin/

clavulanic acid) according to the instructions of the manufacturer.

The incidence of clinical mastitis decreased from 21.0% at the start of the investigation to 4.9% at the end of the investigation. During the same period, the prevalence of subclinical mastitis was reduced from 38.2 to 10.8%. The high cure rates achieved in the present report were similar with the findings of Machado et al. 2008 (8), who reported that CNS are more susceptible to amoxicillin rather than to penicillin, ampicillin and lincomycin.

In the current report, introducing mastitis-control measures based on using an adequate antibiotic therapy, reduced the incidence of clinical mastitis

and the prevalence of sub-clinical mastitis, which is in agreement with previous studies.

In conclusion, the implementation of proper management strategies is helpful to reduce the rate of new intra-mammary infections and to maintain a low SCC. Use of a proper milking technique, the implementation of dry cow therapy, the prompt antibiotic treatment of clinical cases and culling of chronically infected cows based on the measurement of the SCC, reduces the incidence of subclinical and clinical mastitis in the dairy herds.

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