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COAGULASE-NEGATIVE STAPHYLOCOCCI AND THEIR SIGNIFICANCE FOR SUBCLINICAL MASTITIS

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Summary. Coagulase-negative staphylococci are a frequent cause of bovine intramammary infections in modern dairy herds. They have become the most common bacteria isolated from milk samples in many countries. Because of high prevalence of these intramammary infections it has a great impact on bulk tank somatic cell count. The objective of the study was to analyze the impact of coagulase-negative staphylococci intramammary infection on cows somatic cell count and on the potential of these infections to have a major impact on the bulk milk somatic cell count. The study was conducted on two dairy farms with high milk production and increased number of somatic cells in bulk tank milk. Bacteriological examination of milk samples from cows positive on CMT test pointed high prevalence of coagulase-negative staphylococci in cows with secretion disorder in both herds, up to 81.8%. Somatic cell count in these milk samples was also very high and counted over 1 million per milliliter. The highest number of somatic cells was in milk samples from cows with *Staphylococcus aureus*, but prevalence of these infections was very low.

Keywords: coagulase-negative staphylococci, intramammary infection, somatic cells

Introduction. Coagulase-negative staphylococci are a frequent cause of bovine intramammary infections in modern dairy herds. They have become the most common bacteria isolated from milk samples in many countries. Mastitis caused by coagulase-negative staphylococci in most cases remains subclinical, or the clinical signs are mild. For some reason, heifers, and primiparous cows are most susceptible to coagulase-negative staphylococci mastitis. Coagulase-negative staphylococci mastitis increases milk somatic cell count in the infected udder quarter. Very important is trait of coagulase-negative staphylococci mastitis to not causes decreasing in milk production, some authors even have found that cows with this form of mastitis had greater milk production than cows with no udder infection (Piepers et al., 2008). The increase in milk somatic cell count is usually moderate compared with mastitis caused by many other common pathogens, including *Staphylococcus aureus* and streptococci. However, high prevalence of coagulase-negative staphylococci mastitis in a herd can affect the herd bulk milk somatic cell count (Taponen, 2008).

Intramammary infections caused by major mastitis pathogens can reduce the possibility for infection with coagulase-negative staphylococci.

It seems that coagulase-negative staphylococci mastitis is a particular problem in well-managed, high-producing farms, which have successfully controlled udder infections caused by major mastitis pathogens (Myllys and Rautala, 1995).

About half of the cows with coagulase-negative staphylococci mastitis showed some clinical signs, but in most cases the signs were mild. Often only changes in milk appearance, such as clots and flakes, were detected, but sometimes also slight swelling of the affected quarters. Lack of clinical symptoms and changes in milk appearance is making diagnosis of this form of mastitis problematic and demands orderly applying of California Mastitis Test in dairy production herds.

The spontaneous elimination rate of coagulase-negative staphylococci mastitis is generally regarded as high. Some studies have demonstrated spontaneous elimination rates of as high as 60–70% for intramammary infections caused by coagulase-negative staphylococci (McDougall, 1998; Wilson et al., 1999). This process can be supported with regular applying of teat dipping and stricter hygiene on farms which allows achieving god hygienic quality of milk and an optimal number of somatic cells (Boboš et al, 2012).

Therapy of coagulase-negative staphylococci mastitis is bound with antibiotic resistance of these bacteria. Coagulase-negative staphylococci tend to be more resistant than *Staphylococcus aureus* and easily develop multiresistance. The most common resistance mechanism is β-lactamase production, which results in resistance to penicillin G and aminopenicillins. The reported percentage of penicillin resistance for coagulase-negative staphylococci isolated in mastitis was 32% in Finland (Pitkälä et al., 2004).

Materials and methods. The research was conducted on two dairy farms on the territory of Vojvodina providence. The reason for the inception of research was increased somatic cell count in bulk tank milk samples from observed farms. Somatic cell count in bulk tank

milk samples from both farms was between 400,000 and 500,000 per milliliter. Before forming of experimental group of cows a CMT test was performed on all cows in production. From cows with detected secretion disorder milk samples for bacteriology testing were taken.

Before milking cows were prepared for sampling and milk samples were collected as described.

Milk samples for bacteriology were collected aseptically. The udder and especially the teat were cleaned of dirt with a textile cloth moistened with distilled water. After that, the teat apex was cleaned with a cotton swab moistened with antiseptic solution. Samples were then stored in mobile refrigerator and transported to laboratory for further analysis.

In the laboratory, ten microlitres of milk were streaked on blood agar and incubated at 37 °C overnight (18–22 hours). Staphylococci were further identified based on colony morphology, Gram-staining, microscopy, and a catalase test.

Besides milk samples for bacteriology, cumulative milk samples for determination of somatic cell count were also obtained. These samples were conserved using 'azodiol' containing sodium azide in order to prevent decomposition of somatic cells in milk sample.

Determination of somatic cell count in cumulative milk samples was done in Laboratory for raw milk control on Faculty of Agriculture in Novi Sad, by flow cytometry.

Results and discussion. Bacteriological analysis of cumulative milk samples showed significant presence of coagulase-negative staphylococci in samples from both farms in the experiment. Results of the analysis are shown in Table 1.

Table 1 — Bacteriological findings in milk samples

Isolate	*Staphylococcus aureus*	Coagulase-negative staphylococci	Bacteriologicaly negative
First farm	2	30	21
Second farm	4	27	2

Analyzing milk samples from the first farm for presence of bacteria it can be concluded that from total 53 milk samples, coagulase-negative staphylococci were isolated in 30 samples (56.6%). On second farm from total 33 samples coagulase-negative staphylococci were isolated in 27 samples (81.8%). This result is in accordance with findings of Wilson et al. (1997), who claims that coagulase-negative staphylococci are isolated in high percent from the milk of cows with secretion disorder.

Determination of somatic cell count in milk samples showed increased number of somatic cell in milk samples with positive bacteriological findings (Table 2).

Table 2 — Somatic cell count in milk samples with different bacteriological findings

Isolate	*Staphylococcus aureus*	Coagulase-negative staphylococci	Bacteriologicaly negative
First farm	1,560,000/ml	1,135,000/ml	220,000/ml
Second farm	1,209,000/ml	1,298,000/ml	280,000/ml

The highest number of somatic cells was in samples with *Staphylococcus aureus*, but samples with coagulase-negative staphylococci also had very high values for somatic cell count, with average value 1,216,000/ml. Taponen (2008) also claimed that number of somatic cells in milk from infected cows can be highly increased up to above 1 million per milliliter.

Intramammary infections are affecting quality of milk, this can be measured through determination of somatic cell count (Radinović et al., 2014). Coagulase-negative staphylococci are very often isolated from milk and although they were not considered to be important pathogen for mammary gland, latest studies are pointing the significance of these bacteria (Taponen et al., 2006).

Coagulase-negative staphylococci are commonly considered to be teat skin opportunists that normally reside on the teat skin and cause mastitis via ascending infection through the streak canal (Radostits et al., 2007). This finding highlights significance of good udder hygiene and applying of teat dipping in order to prevent penetration of bacteria in mammary gland through teat canal. When applying therapy of coagulase-negative staphylococci mastitis, it is important to consider their resistance through β-lactamase production (Pitkälä et al., 2004). By improving cows management and hygiene, it is possible to support the process of self-healing or spontaneous elimination (MMM, 2003). The spontaneous elimination rate of CNS mastitis is generally regarded as high. Some studies have reported spontaneous elimination rates of about 60–70% (McDougall, 1998; Wilson et al., 1999). Markedly lower rates, 15–44%, have also been reported (Rainard and Poutrel, 1982; Timms and Schultz, 1987). In Finland and in the other Nordic countries, the policy is to avoid unnecessary use of antimicrobials in animal husbandry (MMM, 2003). Subclinical and mild clinical mastitis caused by CNS is usually left untreated, the rationale being that CNS will be eliminated spontaneously.

Conclusion. Coagulase-negative staphylococci are very significant cause of subclinical mastitis in dairy cows. Their effect on milk quality is measured through increasing of somatic cell count. Clinical form of mastitis is rare and decreasing of milk production is irrelevant. Good hygiene is very important for control of these agents allowing the spontaneous elimination from udder.

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