

## OCCURRENCE OF MASTITIS PATHOGENS AT DRY OFF PERIOD AND AFTER CALVING IN DAIRY COWS

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### Summary

The aim of the work was to study the occurrence of mastitis pathogens before and after calving in the same dairy cows. The Holstein cows suspicious on subclinical mastitis (positive California Mastitis Test) were sampled at quarter level under practical farm with high level of milk yield (11,278 kg). The cows were treated with antibiotics (Cefquinomum) before drying. In total 84 samples before drying and 107 samples after calving from the same dairy cows were collected. The samples were cultured on blood agar (MkB Test as, Rosina, SR). MALDI-TOF MS (Bruker Daltonics, Germany) was used to identify mastitis pathogens. Bacteriologically positive (BP) samples from dairy cows before drying were found in 35% of the milk samples. The most frequent pathogens in BP milk samples were coagulase-negative staphylococci (CNS) (77.38%). The most common CNS was *Staphylococcus (S.) xylosus* (32.14%). *S. aureus* was detected in 5.95% of BP samples. After calving, we found BP samples in 14.02% of dairy cows. The most common pathogens in milk samples were CNS (10.39%). *S. aureus* was detected in 0.94% of BP samples. Antibiotic treatment during the drying period clearly reduced the occurrence of CNS and *S. aureus* in dairy cows at the beginning of lactation.

**Keywords:** dairy cows, mastitis, pathogens

### Introduction

The quality of milk may be evaluated by measurement of parameters that indicate both its suitability for consumption or processing into dairy products, and the health status of the cow or herd producing the milk. The principal parameter routinely used internationally in this context is the somatic cell count (SCC) of milk, which come from the blood and the epithelium of the udder represented number of cells per millilitre of milk (Kelly et al., 2011). The principal parameter routinely used internationally in this context is the somatic cell count (SCC) of milk, or number of white blood cells per millilitre of milk. The quality of milk may also be assessed through measurement of the microbiological population, either through determination of the total bacterial count (TBC) or the presence of specific types of bacteria.

Mastitis have enormous economic importance, especially in connection with the adverse effect of mastitis in dairy cows to produce milk and its marketability, the decommissioning of the milk supply, treatment, change of breeding values and culling (decommissioning) cows. They talk about them even as the most expensive disease of dairy cows (Huijps et al., 2008).

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Milk somatic cell count is a key component of national and international regulation for milk quality and an indicator of udder health and of the prevalence of clinical and subclinical mastitis in dairy herds (Regulation (EC) No. 853/2004 laying down specific hygiene rules for food of animal origin, according to which the somatic cells count in 1 ml of milk  $\leq 400\ 000$ ).

The dry period is a very important period for the dairy cow. It provides the cow with an opportunity to care for the growth of the calf without producing milk and it allows for regeneration of the mammary epithelial tissue before the next lactation. Without the dry period, milk production per day will be reduced while transition problem will not disappear. Dairy cows with a short dry period (10- to 40-days) produced significantly less milk in the following lactation than cows with a 40- to 60-day dry period (Watters et al. 2008). Adequate proliferation and differentiation of mammary secretory epithelium during the non lactating period are essential for optimal synthetic and secretory function in the subsequent lactation, and hence the duration of the non-lactating interval is closely related to milk production (Schukken et al., 2011).

In dairy cows, besides the use of antibiotics in the treatment of clinical and subclinical mastitis during lactation, the use of antibiotics for intramammary use at the time of drying among lactations is also used, but without the proper diagnosis of mastitis, with application of antibiotics in all teats and generally with association with internal sealant, acting as mechanical barrier against environmental agents in the pre-labour (Higgins et al., 2017, Scherpenzeel et al., 2017).

Legislation on the use of antibiotics in disease prevention is increasingly restricted in the area of animal production, including the treatment of antibiotics in the drying period (Kuipers et al., 2016, Piddock, 2016). There is a concern of decreasing the use of antibiotics in the drying of dairy cows, treating only the teats with intramammary infections with the previous diagnosis (Lopes et al., 2019).

The aim of the work was to study the occurrence of mastitis pathogens before and after calving in the same dairy cows.

### ***Material and Methods***

The dairy cows were housed in free housing system. Animals were milked out two times a day in 2 x 10 herringbone milking parlour. The parlour was equipped with automatic devices for automatic cluster removal. The milking routine consists from udder washing with water from hose, cleaning with towel and fore-stripping. Antibiotics dried up. Holstein dairy cows achieved a milk yield of 11,278 kg in 2021. A total of 240 milk samples collected at the level of the udder quarters of dairy cows were analyzed. 27.92% of the samples were evaluated as contaminated. Bacteriological positive samples were detected in 35% of milk samples. The Holstein cows suspicious on subclinical mastitis (positive California Mastitis Test) were sampled at quarter level for mastitis pathogens detection immediately before drying (84 samples) afterwards the cows were treated with antibiotics (Cefquinomum) on fifth day after calving the same dairy cows were sampled (107 samples). Milk samples were incubated on blood agar for 24 hours at 37 degrees Celsius and re-evaluated after 48 hours incubation. Milk sample was classified as positive on subclinical mastitis if one and more colony-forming unit (CFU) of major pathogens was grown. For other pathogens were samples classified as positive on subclinical mastitis if we recorded the grown five and more colony-forming unit. The grown colonies were identified using laboratory tests and Maldi tof ms.

### Results and Discussion

The dry period is probably the time that the mammary gland is most susceptible to an intramammary infection. During the involution phase, the gland is full of milk and therefore quite susceptible to infection. For this reason, the method of dry-off and the use of dry-off antibiotics are essential to prevent infections in the early dry period. Several studies have shown that a lower milk production at dry off is associated with fewer infections in the early dry period (Schukken et al., 2011).

Reported rates of new infection in the dry period in cows not treated with antibiotics at dry off range from 3.8 to 35.1% of quarters with an average of about 10% (Robert et al. 2006).

At present, “environmental” mastitis caused by pathogenic bacteria that easily survive in the environment, especially coagulase-negative staphylococci, dominate in ruminants.

In second place are infectious mastitis caused by the bacteria *Streptococcus agalactiae* and *Staphylococcus aureus*, which spread from dairy cow to dairy cow during milking, using a milking machine, and were largely eliminated by preventive measures.

Bacteriologically positive (BP) samples from dairy cows before drying were found in 35% of the milk samples. The most frequent pathogens in BP milk samples were coagulase-negative staphylococci (CNS) (77.38%). The most common CNS was *Staphylococcus (S.) xylosus* (32.14%, tab.1).

*S. aureus* was detected in 5.95% of BP samples after calving, we found BP samples in 14.02% of dairy cows. The most common pathogens in milk samples were CNS (10.39%).

Table 1 Representation of identified pathogens in milk samples

Pathogens	Occurrence (%)	
	Before	After
<i>S. aureus</i>	5,95	1,30
<i>S. xylosus</i>	32,14	4,55
<i>S. chromogenes</i>	29,76	4,55
<i>S. epidermidis</i>	7,14	-
<i>S. haemolyticus</i>	5,95	-
<i>Str. uberis</i>	4,76	-
<i>Corynebacterium amycolatum</i>	3,57	-
<i>Serratia ureilytica</i>	3,57	-
<i>S. arlettae</i>	2,38	-
<i>Enterococcus faecalis</i>	1,19	-
<i>Str. lutetiensis</i>	1,19	-
<i>Citobacter koseri</i>	1,19	-
<i>Serratia rubidae</i>	1,19	-
<i>Escherichia coli</i>	-	0,65
<i>Str. sciuri</i>	-	0,65

*S.* - *Staphylococcus*, *Str.* - *Streptococcus*

The selective treatment of teats in dry dairy cows has advantages over Blanket Dry Cow Therapy by reducing the indiscriminate use of antibiotics, avoiding bacterial resistance, ensuring better milk quality and greater food safety. Antibiotics should only be used for teats with subclinical mastitis, with the microbiological culture at the end of lactation performed by fourth individual mammary (Lopes et al., 2019). As the prevalence of subclinical mastitis in the herd, the cow's milk yield, and parity before drying off influence the estimates, these factors could be taken into account when choosing (a) SCC threshold(s), for example, when implementing a selective dry cow approach on the farm (Lipkens et al., 2019). Krattley-Roodenburg et al., 2021 were showed, that using dip or spray after the last milking should be recommended, whereas low-SCC cows with previous udder health issues might receive antimicrobial dry cow treatment in contrast to the current guideline. The housing of dry cows seems influential but needs further study.

### **Conclusions**

Antibiotic treatment during the drying period clearly reduced the occurrence of CNS and *S. aureus* in dairy cows at the beginning of lactation.

In conclusion for the dairy farmers is the biggest problem of rapid identification of “problem cows” and determine the pathogens that cause inflammation of the mammary glands. Increased SCC had at the amount and contents of milk the most significant effect, either the quantity or quality of milk. Knowledge of the negative effects of these factors can help dairy farmers in preventing the occurrence and spread of mastitis. Training of employees in this area is important.

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## POJAVNOST UZROČNIKA MASTITISA U RAZDOBLJU SUHOSTAJA I NAKON TELJENJA

### Sažetak

Cilj rada bio je utvrditi pojavu uzročnika mastitisa u vrijeme suhostaja i nakon teljenja. Krave Holštajn pasmine visoke razine proizvodnje mlijeka (11.278 kg) sa sumnjom na prisutnost uzročnika mastitisa (pozitivni Kalifornija mastitis test) u barem jednoj četvrti uključene su u istraživanje. Krave su prije zasušenja tretirane antibiotikom (Cefquinomum). Ukupno je prikupljeno 84 uzoraka mlijeka krava prije zasušenja i 107 uzoraka krava nakon teljenja. Uzorci su kultivirani na krvnom agaru (MkB Test kao Rosina, SR). MALDI-TOF MS (Bruker Daltonics, Germany) je korišten za identifikaciju uzročnika mastitisa. Bakteriološki pozitivni uzorci utvrđeni su kod 35 krava prije zasušenja. Najčešći uzročnik mastitisa utvrđen u bakteriološki pozitivnim uzorcima mlijeka krava prije zasušenja je bio koagulaza negativno stafilokok (CNS) (77,38 %). Najčešće utvrđeni CNS je bio *Staphylococcus (S.) xylosus* (32,14 %) dok je *Staphylococcus aureus* izoliran iz 5,95 bakteriološki pozitivnih uzoraka. Nakon teljenja, utvrđeno je 14,02 % bakteriološki pozitivnih uzoraka mlijeka krava. Najčešći patogeni u uzorcima mlijeka bili su CNS (10,39 %). *S. aureus* je otkriven u 0,94 % bakterioloških uzoraka. Liječenje antibioticima tijekom razdoblja zasušenja jasno je smanjilo pojavu CNS -a i *S. aureus* kod mliječnih krava na početku laktacije.

**Ključne riječi:** mliječne krave, mastitis, uzročnici mastitisa

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