

## Aetiology and prevalence of bovine intramammary infection at drying off

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### ABSTRACT

The aim of this research was to determine the etiology and prevalence of udder infection in dairy cows before drying off on dairy farms in Croatia. Research was carried out on 6 dairy farms in Croatia. Milk samples were taken in sterile plastic tubes during the last milking prior to drying off. Bacteriological examination of the milk samples produced 54 positive samples; the prevalence of intramammary infection (IMI) was 22.5%. The most common pathogens of IMI prior to drying off were *Streptococcus* spp. Lancefield group D (7.79%) and *Staphylococcus aureus* (6.56%). Other pathogens were coagulase-negative staphylococci (2.87%), *Pseudomonas* spp. (2.46%), *Streptococcus agalactiae* (2.05%), and other microorganisms (1.23%). In this research, a higher IMI prevalence in the rear quarters ( $P = 0.03$ ) compared to the front quarters was observed. There was no significant difference in the prevalence of infection ( $P = 0.07$ ) when comparing the right and left quarters. A significantly higher prevalence of infection caused by *Streptococcus* spp. Lancefield group D ( $P = 0.04$ ) and environmental pathogens ( $P = 0.02$ ) was noted in the rear quarters. These results proved that environmental microorganisms were the most common pathogens of IMI, prior to drying off, in dairy cows in Croatia.

**Key words:** cow, mastitis, intramammary infection, prevalence, etiology

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### Introduction

Mastitis, in spite of great scientific efforts to reduce it, still remains the most expensive disease in dairy cows. The problem of mastitis should be considered as a

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problem of milk herds in general and cattle breeding in the country as a whole (CERGOLJ and TOMAŠKOVIĆ, 2003).

The most important pathogens of bovine mastitis are *Streptococcus*, *Staphylococcus*, *Escherichia coli* and other coliform microorganisms (RADOSTITS et al., 2007).

The importance of drying off in mastitis control was recognized long ago (SMITH et al., 1967), and was proved by using antibiotics during the drying off period, incorporated in the mastitis five point plan (KINGWILL et al., 1970). The most important change in the epidemiology of bovine mastitis over the past decade has been the rise in the importance of environmental pathogens which cause clinical mastitis, in comparison to contagious pathogens. Studies worldwide have confirmed that the mammary gland in the dry period is susceptible to environmental microorganisms (BRADLEY and GREEN, 2001; BERRY and HILLERTON, 2002; BRADLEY and GREEN, 2002).

Recently published references have confirmed that environmental microorganisms are the most important pathogens of IMI during the dry period in the majority of herds with a moderate somatic cell count (BRADLEY et al., 2007).

There is no reliable data available in recent, peer-reviewed literature on the prevalence and etiology of intramammary infection (IMI) prior to drying off in dairy cows in Croatia. The purpose of this study was to estimate the prevalence of IMI in a random selection of dairy herds in Croatia, to identify the pathogens prior to drying off and to compare the results with those published recently in scientific papers.

### Materials and methods

In this research project, 61 dairy cows were selected from six farms, randomly distributed in the Zagreb and Varaždin areas. Herd sizes ranged from a minimum of 9 to a maximum of 24 cows. The breed composition was Holstein Frisian, Simmental, and Simmental crossbreed. On the selected farms, the cows were housed in cowsheds, or in a combination of cowsheds and pastures. The average milk production was 3.000 to 6.000 litres per cow per standard lactation (305 days). The basic components of feed were hay, fresh cut grass, silage or haylage (corn, barley and wheat) and TMR.

Samples for bacteriological culture from each quarter, prior to drying off, were collected aseptically in sterile 10 mL tubes, without additives, according to the National Mastitis Council (HOGAN et al., 1999) and kept at 4 °C during transport. Samples were analyzed within 12 hours of collection.

*Microbiological culturing.* 0.01 mL of milk sample was cultured in one quarter of a Petri dish containing esculin blood agar. Incubation was at 37 °C for 24 hours; after that control was performed. The remains of the samples were stored under refrigeration until the end of incubation. If there was no bacterial colony growth in one quarter of the Petri

dish, 0.1 mL of milk was again incubated in one half of a Petri dish. Bacterial colonies were determined 24, 48 and 72 hours after incubation. Morphological characteristics (shape, size and structure of colonies) and physiological features (pigment formation, generate CAMP phenomena, esculin disintegration, ability to coagulate rabbit plasma, Gram staining, catalase test) were assessed.

*Statistical analysis and modelling:* To establish the relationship between a binary outcome variable and group of predictor variables, the logistic regression model (PROC LOGISTIC) was used according to SAS 9.1.3. Software (SAS Institute Inc., 2002-2003). The outcome variable was infection of a quarter with the specific microorganism found in the milk sample (positive test = 1). The dichotomous predictor variables were quarter level (rear vs. front and right vs. left quarters). For this analysis, an odds ratio and Chi-square probability values were presented.

## Results

In this research, 244 milk samples were tested (all four quarters of 61 cows). In 54 samples, mastitis caused by bacteria was found.

Table 1. Results of bacteriological findings

Bacteriological finding in quarters	N	%
Negative	188	77.05
<i>Staphylococcus aureus</i>	16	6.56
<i>Streptococcus</i> spp. Lancefield group D	19	7.79
Coagulase-negative staphylococci	7	2.87
<i>Streptococcus agalactiae</i>	5	2.05
<i>Pseudomonas</i> spp.	6	2.46
<i>Escherichia coli</i>	2	0.82
<i>Streptococcus dysgalactiae</i>	1	0.41
Total	244	100

The most common pathogens of IMI were *Streptococcus* spp. Lancefield group D (7.79%) and *Staphylococcus aureus* (6.56%). Coagulase-negative staphylococci (2.87%), *Pseudomonas* spp. (2.46%), *Streptococcus agalactiae* (2.05%) were also very important pathogens of IMI, while 1.23% of IMI was caused by other microorganisms (Table 1).

The prevalence of *Staphylococcus aureus* infection was not significantly different between the quarters, or in quarters infected with *Streptococcus agalactiae*. In testing infected quarters for *Streptococcus* spp. Lancefield group D ( $P = 0.04$ ) and other environmental ( $P = 0.02$ ) pathogens, a significantly higher rate of infected quarters was noted.

Table 2. Prevalence of intramammary infections by quarters

Prevalence of intramammary infections	Estimate	SE	OR <sup>1</sup>	CI <sup>2</sup> (95%)	P
Rear/front quarters	0.66	0.31	1.93	1.05-3.56	0.03
Right/left quarters	-0.56	0.31	0.57	0.31-1.05	0.07
Rear/front quarters ( <i>Staphylococcus aureus</i> )	-0.27	0.52	0.76	0.26-2.12	0.61
Rear/front quarters ( <i>Streptococcus</i> spp. Lancefield group D)	1.11	0.54	3.03	1.06-8.70	0.04
Rear/front quarters (contagious pathogens)	-0.31	0.46	0.73	0.27-1.80	0.49
Rear/front quarters (environmental pathogens)	1.02	0.42	2.76	1.22-6.28	0.02

<sup>1</sup>OR = Odds ratio; <sup>2</sup>CI = Confidence Interval

## Discussion

This is the first research into intramammary infections, prior to drying, off in dairy cows in Croatia. In research of this nature, it is difficult to be sure that results truly represent the target population in the whole of Croatia. Etiologically, the most common pathogens of IMI prior to drying off were *Streptococcus* spp. Lancefield group D (7.79%) and *Staphylococcus aureus* (6.56%). MARIĆ and MAJIĆ (1979), during a five-year period in the mid 1970s, determined the presence of secretion disorders in 15.7% of the quarters examined, which represents 34.9% of the cows examined. The most prevalent isolated pathogen was *Staphylococcus aureus* (21.93%). *Streptococcus* spp. Lancefield group D was isolated in 11.78% and *Streptococcus agalactiae* in 1.2% of the cows examined.

In a survey conducted after the Homeland War in Croatia, TOPOLKO and BENIĆ (1997) examined 250 lactating cows and found pathogens in 20.5% of milk samples. The bacteria isolated were *S. aureus* in 10.8%, *Streptococcus agalactiae* in 2.9%, other *streptococci* in 5.2%, and other pathogens in 1.5% of samples. BENIĆ et al. (2005) reported that contagious pathogens were prevalent in Croatia in 1996, causing 14.6 per cent of IMI, and environmental pathogens were determined in 5.9% of samples. In 2004, contagious pathogens were also prevalent, causing 13.4% of IMI during lactation, whereas IMI caused by environmental pathogens had increased to 8.4% (BENIĆ et al., 2005).

In our research, the prevalence of infection of quarters prior to drying off was 22.95%, and the herd prevalence of IMI was 60.7%. RADOSTITS et al. (2007) proved that, in most countries, the prevalence of mastogenic infection in dairy herds is 50% (positive cows), which means 10% to 25% positive quarters. PAVLAK et al. (2008) indicated that the level of the prevalence of quarter IMI in Croatia in 1996 was 34%; in 2004 it was 28.7%, while

in this research during 2008 and 2009, the prevalence was 23%. It is obvious that the prevalence of mammary gland infection during the last few years has been decreasing, due to better animal housing (cleaner, drier flooring), nutrition and milking hygiene. The prevalence of IMI before drying off was 5% to 28% for all quarters (DINGWELL, 2002).

In our research, the higher prevalence of IMI in rear quarters as compared to front quarters was noted prior to drying off ( $P = 0.03$ ; Table 2). The significantly higher prevalence of IMI in rear quarters is in accordance with previous research (BARKEMA et al., 1997; BERRY and MEANEY, 2006). All these authors noted the significantly higher prevalence of clinically manifested mastitis in rear quarters. The higher prevalence of infection in rear quarters is due to udder morphology. It may also be attributed to higher milk production in the rear udder quarters (LANCELOT et al., 1997). The prevalence of IMI increases with milk production (HOUBEN et al., 1993). Transmission of IMI can occur, not only between infected cows, but also between the cow's own quarters. Because the known contagious pathogens of mastitis, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, are mainly transmitted during milking (LAM et al., 1996), interdependence among the quarters based on transmission is more likely for these pathogens than for pathogens such as *Streptococcus* spp. Lancefield group D, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Escherichia coli* (BARKEMA et al., 1997). Similar results were obtained in our research for the period prior to drying off. During testing of infected quarters for *Streptococcus* spp. Lancefield group D ( $P = 0.04$ ) and other environmental ( $P = 0.02$ ) pathogens, a significantly higher rate of infected quarters was noted (Table 2). The results obtained support the thesis (BARKEMA et al., 1997) that transmission of environmental pathogens is less likely between the quarters of the same cow. The primary source of these pathogens is the environment. Factors that affect the increased presence of microorganisms are overcrowding, wet or scarce bedding, milking wet udders, poor udder preparation, and functionally and technically inadequate milking machines (BAČIĆ, 2009).

### Conclusion

The results of this research suggest that the prevalence of quarter infection prior to drying off in dairy herds in Croatia is probably about 23%, whilst in herds it affects 60 of every 100 cows, higher than previously thought. The results of this research proved that environmental pathogens are the most important pathogens of IMI, prior to drying off, in dairy cows in Croatia.

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**SAŽETAK**

Cilj istraživanja bio je utvrditi etiologiju i prevalenciju infekcije vimena mliječnih krava prilikom zasušivanja. U istraživanje je bila uključena 61 muzna krava sa šest različitih, nasumično odabranih farmi iz dijela Zagrebačke i Varaždinske županije. Uzorci mlijeka za mikrobiološku pretragu uzeti su u sterilne plastične epruvete prije posljednje mužnje u laktaciji. Bakteriološkom pretragom mlijeka dobili smo 54 pozitivna uzorka, odnosno prevalencija infekcije iznosila je 22,5%. Najveći broj intramamarnih infekcija (IMI) prije zasušivanja uzrokovali su *Streptococcus* spp. serološke skupine D (7,79%) i *Staphylococcus aureus* (6,56%). Od ostalih uzročnika mogu se izdvojiti *Staphylococcus* spp. (2,87%), *Pseudomonas* spp. (2,46%), *Streptococcus agalactiae* (2,05%) te ostali mikroorganizmi (1,23%). U provedenom istraživanju zabilježena je veća prevalencija IMI u stražnjim ( $P = 0,03$ ) u odnosu na prednje četvrti. Uspoređujući desne četvrti u odnosu na lijeve nije uočena značajna razlika prevalencije infekcije ( $P = 0,07$ ). Prilikom testiranja broja inficiranih četvrti na *Streptococcus* spp. serološke skupine D ( $P = 0,04$ ) kao i svih ostalih okolišnih uzročnika ( $P=0,02$ ) zabilježen je značajno veći broj inficiranih stražnjih četvrti. Rezultati istraživanja pokazuju da su mikroorganizmi iz okoliša najznačajniji uzročnici IMI prije zasušenja krava.

**Cljučne riječi:** krava, mastitis, intramamarna infekcija, prevalencija, etiologija

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