### Udder-related risk factors for clinical mastitis in dairy cows

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A cross-sectional longitudinal survey was carried out between February 2009 and January 2010 to evaluate udder-related risk factors for clinical mastitis occurring in dairy herds. Data were used from three dairy farms. Research was divided into four calendar seasons. Cows with clinical mastitis were detected by clinical examination of the udder and determination of abnormalities in milk. Udder level variables, conformation characteristics of udder and teats and teat end to floor distances were included in the logistic regression analysis. The estimated incidence risk for CM in the population of dairy cows observed was 85.02 cases per 100 cowyears at risk. The relative risk of CM was lower for primiparous cows, and increased with further parity. The median number of days in milk at diagnosis was 108 days, ranging from a median of 55 to 150 days in lactation. The rear udder quarters had a higher risk of CM incidence compared to the front udder quarters. A Chi square test revealed that farm management and cow parity were significantly connected with incidence of clinical mastitis. Spring was the season with the highest percentage of diagnosed cases of CM, with the exception of farm A, where Fall was the season with the highest percentage of cases. All udder level factors entered in the models were significantly linked with the occurrence of CM. The odds ratio of CM increased significantly as udder morphology worsened, teat ends were flat and the distance from teat ends to floor decreased. It was concluded that conformation udder traits could be used for the genetic selection of dairy cows for mastitis resistance.

Key words: dairy cows, clinical mastitis, risk factors

#### Introduction

Mastitis is one of the most devastating diseases in the dairy industry. Udder health disorders cause profound economic loss and have a major influence on dairy cows' welfare and productivity (HALASA et al., 2007; HOGEVEEN et al., 2011).

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The incidence and prevalence of clinical mastitis (CM) reported in the literature vary considerably, due to differences in definitions of the disease, or the criteria used for including cases (SARGEANT et al., 1998; SHPIGEL et al., 1998). The incidence of CM also varies considerably by country or region where surveys have been carried out (SVILAND and WAAGE, 2002).

The occurrence of mastitis in dairy herds results from a complex interaction between the host, environment and agent. Generally, the most common risk factors for CM in dairy herds can be divided in two groups: individual cow risk factors and risk factors from the environment. Many authors report risk factors for CM associated with farm management, hygiene management, the breeding environment, milking technology, feeding, the calving season and preventive health management (VAN DORP et al., 1999). In an individual herd, cow factors are responsible for the differences among cows in contracting CM. A great number of individual cow-specific risk factors for CM have been identified, including breed, parity, period of lactation, udder and teat morphology, age at first calving, milk leakage, udder edema, milk production, number of milk somatic cells and reproductive disorders (PEELER et al., 2000; NYMAN et al., 2007; VALDE et al., 2007).

All these studies were conducted in different countries under varying conditions, which may explain the different results, and no specific studies on risk factors for CM have been performed under the climatic, housing and dairy breed conditions of Macedonia.

The aim of this study was to identify important risk factors for CM in high-producing dairy herds. The identification of risk factors is important for the design of mastitis control programs in dairy herds.

#### Materials and methods

A repeated, cross-sectional longitudinal survey was carried out to evaluate risk factors for CM occurring in dairy herds. The data were from three dairy farms located in the Republic of Macedonia. Selection criteria were set to include a reasonable number of milking cows in target dairy farms (n≥50) and the cows had high milk production (a daily milk yield per cow of more than 20 kg). The farms differed in terms of systems and rearing technology, milking technology, size of herd, hygiene management and health management. The management practice on farm A was production in tie-stalls. Milking was performed by a transferable milking system. Pre-milking and post-milking hygiene measures were not practiced. The management practice on farm B was production in a loose-housing system with an open shed, on deep bedding. Milking was performed in a milking parlour. Pre-milking and post-milking hygiene measures were practiced constantly. The management practice on farm C was production in a loose-housing system with an enclosed shed. Milking was performed in a milking parlour. Pre-milking and post-milking hygiene measures were practiced occasionally.

The annual observation period for each cow in lactation was from 1 February 2009, or from the day of parturition when the cow entered the herd, to 31 January 2010, or the day of removal from the herd. The total number of cow-years at risk was the sum of days in lactation for all cows divided by 365. A cow in lactation contributed to the sum of cow-years from the day of beginning the trial or the day of parturition within the trial period, until the end of the trial or day on which it was dried off or culled. Parity was calculated from the number of consecutive lactations. The ages of the cows observed were from the first (1) to the sixth or more lactation  $(6 \ge)$ .

Data for each cow number, parity number and associated calving dates were extracted from the reproductive board. The cows included in the study were selected according to calving dates and lactation periods.

The research was divided into the four seasons of the year. The seasons were defined as spring (March to May), summer (June to August), fall (September to November) and winter (December to February).

The incidence of new cases of CM was recorded daily by herdsman, according to ordinary clinical methods under normal field conditions. Cows with CM were detected by clinical examination of the udder (rubber, tumour, colour, dolour and *function laesa*) and determination of abnormalities in milk (presence of watery milk, flakes, clots, blood, pus, discoloured milk, etc) and disorders in the general health of the animal. Cases of CM were recorded at cow and quarter levels. The calving date was used to estimate median days in milk (DIM) per occurrence of CM case (DIMCM) by using data from the reproductive board.

The risk of CM incidence was calculated as the number of cases of the disease per 100 cow-years at risk. Within the same lactation, to distinguish two consecutive cases of CM, the lag time of nine days was used, that is, four days of antibiotic treatment of the infected quarters of the mammary gland, another four days during which antibiotics continue to be present in milk (during this period, the milk was discarded milk), and the ninth day, when there were no abnormal changes in the milk (SVILAND and WAAGE, 2002).

The risk factors monitored had categorical and continuous values. The following possible risk factors for CM were monitored:

classification of udder conformation

classification of udder teat conformation

measurement of the distance from teat end to floor.

The udder conformation of each cow was classified according to the criteria described by DENTINE and McDANIEL (1984) and SEYKORA and McDANIEL (1985). There were five scoring categories for:

position of front udder quarters

position of rear udder quarters

strength of the ligamentum suspensor mamma.

The conformational characteristics of udder teats (papila mammae) were described using scoring system, separately for:

teat tip shape, two scoring categories, according to SLETTBAKK et al. (1995)

teat shape and placement, five scoring categories, according to SAPP et al. (2003).

The distance between the floor and teat tip was measured in centimeters, separately for:

shortest distance from front teat tips to floor, according to SEYKORA and McDANIEL (1985) and SLETTBAKK et al. (1995)

shortest distance from rear teat tips to floor, according to SEYKORA and McDANIEL (1985) and SLETTBAKK et al. (1995).

Initially, to avoid linearity in regression coefficients, which is an important assumption underlying the logistic regression model, the variables for distance were categorized in three scoring categories. The final variable structure is given in Table 3.

During the survey period, measurements of udder and teat morphology were repeated three times, depending on the lactation phase: early (1-60 days in milking-DIM), mid (61 to 120 DIM) and late lactation (more than 120 DIM). All measurements and data collection were made by same person in exactly the same way.

Statistical procedures were conducted in SPSS 14.0 for Windows. The association between farm management, cow parity and CM incidence was measured by ratio of risks and their significance estimated by Chi-square. In this case, the relative risk of CM was used to compare the incidence of CM among cows in a particular farm or lactation with those in another farm or lactation. The same method was used to estimate the relative risk of CM between cows with different udder and teat conformation characteristics. Maximum likelihood multivariable logistic regression was used to model the relationship between independent variables and CM. Associations between the dependent variable and each of the potential risk factors were first screened in a univariable analysis using Chi-square tests and non-parametric statistical methods such as the Kruskal-Wallis test. Variables with P≤0.25, provided that there was no collinearity (r<0.70) between variables, were then considered for further analysis. Collinearity between the potential risk factors was assessed pair-wise by calculation of Spearman rank correlations. Variables with a high collinearity (r>0.70) were not entered in the model at the same time, and if collinear variables were significantly associated with the outcome, separate models, each containing one of the variables, were tested and the model with the best fit was kept. Variables were removed from this model using forward stepwise selection (likelihood ratio). The inclusions of interactions in the final multivariable models resulted

in no statistically significant influences and were therefore omitted. In this respect, two separate regression models were undertaken. The dependent variable in the first analysis was the binary value of observed cases of CM in front udder quarters, which showed a difference between cows with cases of CM distributed in the front quarters and healthy cows, or cows with cases of CM distributed in the rear udder quarters. The opposite, dependent variable in the second analysis was the binary value of observed cases of CM in rear udder quarters which showed a difference between cows with cases of CM distributed in the rear quarters and healthy cows, or cows with cases of CM distributed in the front udder quarters. Cows which had calved prior to 90 days before the start of the field trial, and cows in lactation for less than 30 days prior to the end of the trial period were excluded from the final statistical model. In the models, logistic regression defines categorical variables as deviation of each category of the predictor variable except the reference category, compared to the overall effect. The first category for each variable was taken as the reference category. The fit of the models was evaluated using Hosmer-Lemeshow goodness-of-fit statistics (HOSMER and LEMESHOW, 2000). Influential points and outliers were identified by Cook's distance values. Regression coefficients were estimated by maximum likelihood equations. Confidence limits for the coefficients were computed by the profile likelihood method. The P values (two tailed) for coefficients were based on the Wald statistic. Odds ratio (OR) and 95% confidence limits for OR for each of the variables, adjusted for the effects of other variables in the model, were calculated as the antilogarithm of the estimated coefficient and its confidence limits.

#### Results

The data for distribution of cow parity in the studied population of dairy cows are presented in Fig 1.

The annual incidence risk of CM in observed dairy farms, relative risk and median day in lactation when a case of CM was diagnosed are shown in Table 1.

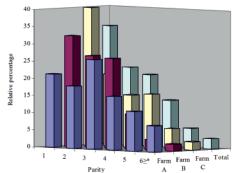


Fig. 1. Parity distribution of dairy cows in study population

Table 1. Annual incidence risk of CM, median day in lactation when case was diagnosed and relative lactation risk of CM in observed dairy farms

			Lactation							
Farm		1	2	3	4	5	6≥	Total		
	Cow-years	20.15	17.13	24.70	14.79	10.91	7.19	94.87		
	CM cases	6	9	8	10	7	5	45		
A	CM incidence	29.78	52.53	32.39	67.61	64.17	69.55	47.43		
	DIMCM	82.50	55.00	73.50	171.50	104.00	97.00	84.00		
	Relative risk	0.57	1.13	0.61	1.55	1.42	1.52	0.52		
	Cow-years	80.08	66.46	65.12	25.11	7.96	5.13	249.85		
	CM cases	108	94	113	53	10	7	385		
В	CM incidence	134.86	141.44	173.54	211.05	125.69	136.55	154.09		
	DIMCM	125.00	150.50	87.00	106.00	98.00	82.00	117.00		
	Relative risk	0.83	0.89	1.18	1.43	0.81	0.88	3.50		
	Cow-years	130.18	68.18	49.29	50.94	19.19	7.90	325.67		
	CM cases	33	38	20	31	10	8	140		
С	CM incidence	25.35	55.74	40.58	60.86	52.11	101.32	42.99		
	DIMCM	112.00	99.00	94.50	102.50	85.00	34.00	108.00		
	Relative risk	0.46	1.41	0.93	1.53	1.23	2.44	0.34		
	Cow-years	230.42	151.77	139.10	90.84	38.05	20.21	670.40		
	CM cases	147	141	141	94	27	20	570		
Total	CM incidence	63.80	92.91	101.36	103.48	70.95	98.95	85.02		
	DIMCM	119.00	126.00	87.00	106.50	102.00	58.00	108.00		
	Relative risk	0.66	1.12	1.26	1.26	0.83	1.17			

The estimated risk of CM incidence in the population of dairy cows observed was 85.02 cases per 100 cow-years at risk. The highest incidence risk was calculated for the cow population on farm B, while the incidence risk was similar on Farms A and C. According to this result, the cows on Farm B had a higher relative risk of CM than the cows on Farms A and C. The relative risk of CM was lower for primiparous cows, and increased with further parity. On all three farms, as lactation numbers increased, the frequency of cows with CM increased, with minor exceptions among cows in their 3<sup>rd</sup> and 5<sup>th</sup> lactations. Considering the entire observed population of dairy cows, the median number of DIM at diagnosis of CM (DIMCM) was 108 days, ranging from a median of 55 to 150 DIM for different parity. A statistically significant association was found between CM incidence and farm management ( $\chi^2 = 370.996$ , df = 2, P<0.001). Similarly, a statistically significant association was found between parity and CM incidence ( $\chi^2 = 32.309$ , df = 5, P<0.001).

Table 2 shows CM incidence distribution between front and rear udder quarters. The rear udder quarters had a higher risk of CM incidence compared with front udder quarters.

Table 2. Annual incidence of CM according to the distribution of cases in front and rear udder quarters

Farms	Front quarters	Rear quarters	Front/rear quarters	CM incidence
A	14.76	24.24	8.43	47.43
В	60.44	69.24	24.41	154.09
С	6.76	26.71	9.52	42.99
Total	27.89	42.21	14.92	85.02

Fig. 2 shows the distribution of CM according to the seasons of the year when cases were diagnosed, expressed as relative percentages of the total incidence risk. Seasonal variations for the occurrence of CM during the year ranged from 4.44% to 40.78% of all cases. Spring was the season with highest percentage of diagnosed cases of CM, with the exception of farm A, where Fall was the season with the highest percentage of cases diagnosed.

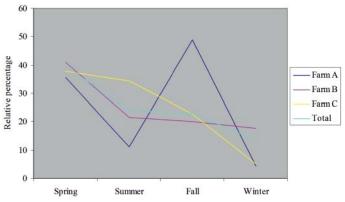


Fig. 2. Distribution of CM during seasons of the year

A description of the variables and levels used in the study is given in Table 3.

Table 3. Description of study variables used in the logistic regression model

Variable	Description	Level
CM	Clinical mastitis in dairy cows	0 = No; 1-Yes
UF	Position of front udder quarters	1 = Moderate length, firmly attached; 2 = Moderate length, slightly bulgy; 3 = Short; 4 = Loose; 5 = Faulty
UR	Position of rear udder quarters	1 = Firmly attached, high, wide; 2 = Intermediate height and width; 3 = low; 4 = Narrow and pinched; 5 = Loose
UL	Strength of ligamentum suspensor mamma	1 = Strong suspensor; 2 = Lack of defined halving; 3 = Floor too low; 4 = Tilted; 5 = Broken suspensor
TF	Front teat tip shape	1 = Semi pointed or pointed; 2 = Semi platform or platform
TR	Rear teat tip shape	1 = Semi pointed or pointed; 2 = Semi platform or platform
PF	Front teat shape and placement	1 = small and squarely placed; 2 = small and inside placement; 3 = mid length and intermediate symmetry; 4 = large and outside placement; 5 = undesirable and wide placement
PR	Rear teat shape and placement	1 = small and squarely placed; 2 = small and inside placement; 3 = mid length and intermediate symmetry; 4 = large and outside placement; 5 = undesirable and wide placement
DF	Shortest distance from front teat ends to floor	1 = (>55 cm); 2 = (50-55 cm); 3 = (<50 cm)
DR	Shortest distance from rear teat ends to floor	1 = (>55  cm); 2 = (50-55  cm); 3 = (<50  cm)

The relative risks for the distribution of CM between cows with different scores for udder and teat morphology are presented in Tables 4 and 5.

The estimation of interdependence between variables in the statistical model was performed using the Spearman rank correlation, shown in Table 6.

A total of nine variables were screened in the initial univariable analysis, and eight of these were significantly associated with the occurrence of CM. One variable had a P-value of  $\leq$ 0.25, and was therefore excluded from the final regression models. To avoid collinearity between variables, two different regression models were taken in consideration separately, for front and rear udder quarters morphology. The results of the final multivariate logistic regression models are summarized in Table 7 and 8.

Table 4. Link between udder and teat conformation scores and CM incidence measured by ratio of risks

		Farm A	Farm B	Farm C	Total
	1	0.59	0.90	0.39	0.98
LID	2	1.79	1.46	1.10	1.03
UF	3	0.56	0.61	1.09	0.67
	4	2.80	1.24	1.89	1.32
	5	2.98	1.47	2.46	1.73
	1	0.47	0.84	0.39	0.94
	2	0.43	1.00	0.47	0.63
UR	3	0.95	0.99	1.01	0.75
	4	1.83	1.06	2.03	1.32
	5	2.51	1.50	2.39	1.86
	1	0.77	0.79	0.41	0.95
	2	0.55	1.08	0.48	0.63
UL	3	0.41	1.15	1.08	0.85
	4	1.80	1.05	2.20	1.39
	5	3.03	1.49	1.97	1.66
TF	1	0.78	0.91	0.59	0.61
11	2	1.27	1.09	1.67	1.62
TR	1	1.23	0.92	0.60	0.69
1 K	2	0.81	1.07	1.66	1.45
	1	0.75	0.83	0.53	1.02
	2	0.54	0.86	0.78	0.68
PF	3	1.58	1.31	1.47	1.37
	4	1.97	1.39	1.88	1.55
	5	3.50	1.23	1.31	1.21
	1	0.58	0.85	0.41	0.98
	2	0.67	0.82	0.81	0.67
PR	3	1.50	1.38	1.59	1.46
110	4	1.76	1.37	1.62	1.46
	5	3.50	1.23	2.65	1.82

A description of the variables and levels used in the study is given in Table 3

Table 5. Mean and standard deviation for distance in centimeters from teat ends to floor

Farm		Distance from front teat ends to floor	Distance from rear teat ends to floor
Α	Healthy cows	$52.41 \pm 5.641$	$52.31 \pm 5.706$
A	Cows with CM	$48.96 \pm 6.616$	$48.82 \pm 6.713$
В	Healthy cows	$53.56 \pm 5.614$	$53.69 \pm 5.789$
В	Cows with CM	$51.52 \pm 5.891$	$51.63 \pm 6.102$
С	Healthy cows	$51.58 \pm 6.048$	$51.29 \pm 6.333$
	Cows with CM	$47.94 \pm 6.539$	$47.29 \pm 6.717$
T-4-1	Healthy cows	$52.12 \pm 5.947$	$51.95 \pm 6.200$
Total	Cows with CM	$50.41 \pm 6.314$	$50.31 \pm 6.584$

Table 6. Spearman rank correlations between variables included in regression analysis

Pearson's	UR	UL	TF	TR	PF	PR	DF	DR
UF	0.811**	0.775**	-0.062**	-0.043	0.234**	0.237**	0.346**	0.358**
UR	1	0.932**	-0.044	-0.026	0.261**	0.263**	0.407**	0.417**
UL		1	-0.035	-0.024	0.277**	0.288**	0.445**	0.456**
TF			1	0.781**	0.061**	0.054*	-0.016	-0.015
TR				1	0.063**	0.061**	0.006	0.003
PF					1	0.915**	0.284**	0.279**
PR						1	0.294**	0.294**
DF							1	0.970**

<sup>\*\*</sup> significant at the P<0,01 level; \* significant at the P<0,05 level. A description of the variables and levels used in the study is given in Table 3.

Two separate regression models were conducted for front and rear udder quarters. A total of 2014 recorded case-observations was available for analysis. In the first analysis, the Hosmer-Lemeshow-  $\chi^2$ - statistic was 3.807 with df 8, P = 0.874. In the second analysis, the Hosmer-Lemeshow-  $\chi^2$ - statistic was 2.984 with df 8, P = 0.935. Both models showed reasonably good fit. All udder level factors entered in the models were significantly linked with occurrence of CM. The OR of front quarters CM cases significantly decreased for level 3 of front udder quarters morphology and significantly increased for level 2 of teat ends shape and distance levels 2 and 3. The OR of rear quarters CM cases significantly increased as rear quarters morphology worsened, teat ends were flat and distance from rear teat ends to floor decreased.

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Table 7. Final logistic regression model for outcome variable observed cases of CM in front udder quarters ( $P \le 0.25$ )

					1		1		
Variable	Level	b	S.E. <sub>b</sub>	Wald	DF	P-value	Odds ratio	959	% CI
UF				24.664	4	P<0.001			
	1	Ref	Ref	Ref			1.00		
	2	-0.196	0.150	1.704	1	0.192	0.822	0.612	1.103
	3	-0.718	0.204	12.392	1	P<0.001	0.488	0.327	0.727
	4	0.091	0.190	0.229	1	0.632	1.095	0.754	1.590
	5	0.467	0.253	3.412	1	0.065	1.595	0.972	2.618
TF					1	P<0.001			
	1	Ref	Ref	Ref			1.00		
	2	0.375	0.088	17.957	1	P<0.001	1.455	1.223	1.730
PF				10.115	4	0.039			
	1	Ref	Ref	Ref			1.00		
	2	0.601	1.567	0.147	1	0.701	1.824	0.085	39.319
	3	1.090	1.567	0.484	1	0.487	2.973	0.138	64.088
	4	0.948	1.581	0.360	1	0.549	2.581	0.116	57.267
	5	-3.524	6.250	0.318	1	0.573	0.029	0.000	6158.55
DF				13.458	2	0.01			
	1	Ref	Ref	Ref			1.00	0.787	0.940
	2	0.204	0.093	4.847	1	0.028	1.126	1.023	1.470
	3	0.217	0.107	4.100	1	0.043	1.243	1.007	1.534
Const.		-2.518	1.567	2.582	1	0.108	0.081		

A description of the variables and levels used in the study is given in Table 3

Table 8. Final logistic regression model for outcome variable observed cases of CM in rear udder quarters ( $P \le 0.25$ )

Variable	Level	b	S.E. <sub>b</sub>	Wald	DF	P-value	Odds ratio	95%	6 CI
UR			D	39.702	4	P<0.001			
	1	Ref	Ref	Ref			1.00		
	2	-0.407	0.145	7.895	1	P<0.001	0.665	0.501	0.884
	3	-0.472	0.135	12.288	1	0.226	0.623	0.479	0.812
	4	0.146	0.120	1.464	1	P<0.001	1.157	0.914	1.465
	5	0.853	0.152	31.556	1	0.005	2.347	1.743	3.161
TR					1	P<0.001			
	1	Ref	Ref	Ref			1.00		
	2	0.275	0.074	13.724	1	P<0.001	1.316	1.138	1.522
PR				9.763	4	0.045			
	1	Ref	Ref	Ref			1.00		
	2	-0.354	0.179	3.901	1	0.048	0.702	0.494	0.997
	3	-0.015	0.181	0.007	1	0.933	0.985	0.690	1.405
	4	-0.375	0.256	2.141	1	0.143	0.688	0.416	1.136
	5	0.778	0.598	1.690	1	0.194	2.176	0.674	7.030
DR				6.779	2	0.034			
	1	Ref	Ref	Ref			1.00	0.787	0.940
	2	-0.215	0.084	6.547	1	0.011	0.807	0.684	0.951
	3	0.144	0.093	2.421	1	0.120	1.115	0.963	1.385
Const.		-0.926	0.172	28.876	1	P<0.001	0.396		

A description of the variables and levels used in the study is given in Table 3

#### Discussion

The control of mastitis in dairy herds depends on the identification and elimination of risk factors associated with the environment, management and the cows. While most risk factors associated with management and the environment are addressed by introducing good management and hygiene practices, selecting dairy cows which are less susceptible to mastitis is also a control measure worthy of consideration.

Longitudinal studies with repeated measurement of mammary status are necessary to identify factors associated with the risk of CM. Unknown or poor case definition, combined with reporting or submission bias, are common problems affecting field studies. Having an investigator live on each of the study farms and observe every animal daily can eliminate these problems. The incidence risk was estimated by taking all cases of CM into account, including repeated cases in the same animal. There might be some doubt as to

whether treatments recorded at short time intervals should be considered as separate cases or repeated treatments of the same case.

The fact that bovine mastitis is a complex disease leads to the assumption that the differences in incidence risk between farms resulted from differences in environmental factors and farm management. Our results correspond with the results of other studies in which farm management had a statistical significant influence on mastitis incidence (FALDELMOULA et al., 2007; GORDON et al., 2013; KANDEMIR et al., 2013), probably due to differences in breeding environments, herd sizes, feeding, milking technology, hygiene management, milk production and genetic variations in the cows' mastitis resistance. An increased rate of clinical mastitis cases was related to the use of conventional management instead of organic farming, ranging from 0 to 1.44 cases per 305 lactating cow-days (RICHERT et al., 2013).

Using data from approximately 2,000 herds in Denmark, BARTLETT et al. (2001) found 46 cases of CM per 100 cow-years. Much lower risks for CM incidence were reported in one study in the Netherlands: MILTENBURG et al. (1996) estimated 13 cases per 100 cowsyears at risk. The mean incidence of CM reported in British dairy herds was 22.8 cases per 100 cow-years (PEELER et al., 2000). Other studies have measured annual incidence as the number of clinical cases per 100 lactations, and figures between 19 and 92 have been reported (SARGEANT et al., 1998). Similar results were recorded by SHPIGEL et al. (1998), when the incidence was 20.8 cases per 100 lactations/years, with variation ranging from 4.2 to 126.8 cases per 100 lactations/years. SVILAND and WAAGE (2002), using different lag times of 4, 9 and 28 days, reported 52, 50 and 47 episodes of CM per 100 cow-years at risk. SHPIGEL et al. (1998) reported that rear quarters had a higher incidence risk (64.7% of quarter cases) than the front quarters. The true incidence is probably substantially higher, because there is considerable underreporting (MORK et al., 2009).

According to the only previous research performed in a dairy herd in the Republic of Macedonia (TRAJCEV and NAKOV, 2010), the prevalence of sub clinical mastitis on rear quarters was 60.61%, and on front quarters39.39%.

The literature data are generally consistent in reporting that cow parity and lactation stage have a significant influence on the prevalence of bovine mastitis (ZERYEHUN et al., 2013), and the risk increases as cow parity increases (SHARMA et al., 2013)

The median number of DIM at diagnosis found in this study was rather similar to the results given in SHPIGEL et al. (1998). According to them, 51.4% of all cases of CM occurred within early and mid lactation, or approximately 117.5 DIM.

It is well established that a favorable association exists between mastitis resistance and several udder type traits. The literature data are generally similar about the genetic correlation between udder depth, udder attachment to the cow's body, milk production and association of these factors with mastitis incidence (SORENSEN et al., 2000; KLEIN et al., 2005; PTAK et al., 2011). The effects of certain unfavorable udder characteristics on mastitis risk are likely to appear when machine milking begins, therefore the initial postpartum period is the best time to study the relationship between udder characteristics and mastitis risk. Several studies have identified udder and teat conformation as risk factors for CM (RUPP and BOICHARD, 2003; BHUTTO et al., 2010; SINGH et al., 2013). According to them, cows with less desirably shaped udders and more udder depth are more susceptible to lesions and contamination by mastitis-causing pathogens which increase the risk of mastitis. However, it should be emphasized that clinical mastitis also influences udder and teat morphological characteristics (KLAAS et al., 2004).

The udder teats are the first line of defense against intra-mammary infection. The probability of mastitis occurring varies considerably between different teat shapes, sizes, teat placement and the morphology of the teat tip (BARDAKCIOGLU et al., 2011). In any case, there is no consensus in the literature about the influence of teat morphology on mastitis occurrence (HAGHKHAH et al., 2011; SINGH et al., 2013).

Some studies have reported that decreasing teat-end to floor distance is a risk factor for CM (SINGH et al., 2013). Also, an increasing proportion of teat lesions, with decreasing teat end to floor distance, is a well-documented risk factor for mastitis (BHUTTO et al. 2010).

This prospective longitudinal study has shown that individual cow factors, along with farm management, are important in influencing the risk of CM during lactation, and these factors indicate different susceptibilities to CM from animal to animal. The conformation udder traits are strong arguments that can be used to improve udder health. Therefore, it has been suggested that selection of cows with desirable udder and teat morphology might help reduce the incidence of mastitis and improve milk quality (JUOZAITIENE et al. 2006).

#### Conclusion

If farmers want to manage successful programs for mastitis eradication in dairy herds, then selection for improving udder and teat conformation traits must be a part of such programs.

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# NAKOV, D., S. HRISTOV, S. ANDONOV, M. TRAJCHEV: Rizični čimbenici vimena važni za pojavu kliničkog mastitisa u mliječnih krava. Vet. arhiv 84, 111-127, 2014.

#### SAŽETAK

Između veljače 2009. i siječnja 2010. provedeno je presječno longitudinalno istraživanje s ciljem procjene vimena kao rizičnog čimbenika kod pojave kliničkog mastitisa u stadima mliječnih krava. Korišteni su podatci s tri farme, a istraživanje je podijeljeno u četiri kalendarske sezone. Krave s kliničkim mastitisom otkrivane su uz pomoć kliničkog pregleda vimena i utvrđivanjem abnormalnosti u mlijeku. Vime, konformacija vimena i sisa, te udaljenost od vrha sisa do poda uključeni su kao varijable u analizu logističkom regresijom. Procijenjeni rizik incidencije za klinički mastitis u promatranoj populaciji mliječnih krava bio je 85,02 slučaja na 100 krava s rizikom. Relativni rizik za klinički mastitis bio je niži kod prvotelki i povišen kod višetelki. Medijan za vrijeme postavljene dijagnoze iznosio je 108 dana, uz granične vrijednosti od 55 do 150 dana laktacije. Stražnje četvrti vimena imale su veći rizik incidencije za klinički mastitis u usporedbi s prednjim četvrtima. Hi-kvadrat testom utvrđeno je da management farme i redni broj telenja krave pokazaju signifikantnu povezanost s incidencijom kliničkog mastitisa. Proljeće je bila sezona s najvišim postotkom dijagnosticiranih slučajeva kliničkog mastitisa, uz izuzetak farme A na kojoj je to bila jesen. Svi čimbenici koji se odnose na vime uključeni u model bili su signifikantno povezani s pojavom kliničkog mastitisa. Omjer izgleda za klinički mastitis signifikantno je porastao s pogoršanjem morfologije vimena, vrhovi sisa bili su pločasti, a udaljenost od vrha sisa do poda je opadala. Zaključeno je da obilježja konformacije vimena mogu biti korištena u genetskoj selekciji mliječnih krava s ciljem veće otpornosti na mastitis.

Ključne riječi: mliječne krave, klinički mastitis, rizični čimbenici