

ABSTRACT

Clinical mastitis (CM) is the most prevalent health disorder in dairy farms that causes poor milk quality and decreased milk yield. A one-year cross-sectional longitudinal survey was carried out to evaluate udder-related risk factors for CM occurring in dairy herds. The databases were used from three dairy farms. The 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. The quarter milk samples were screened using California mastitis test (CMT) for the detection of abnormal milk secretion (AMS) and microbiological methods for the detection of intramammary infections (IMI). The isolated mastitis pathogens were grouped as contagious: *Streptococcus agalactiae* and *Staphylococcus aureus*; or environmental: *Enterococcus* spp., *Pseudomonas aeruginosa*, *Escherichia coli* and *Aspergillus niger*. The annual prevalence of clinical mastitis was 34.13% at the cow level, and 30.07% at the lactation level. The annual lactation incidence risk (LIR) for the entire population of cows was 45.86%. The prevalence of clinical mastitis and LIR increased with cow parity. Management of farms, the season of calving, and udder level factors entered in the regression model were significant-

ly linked with the occurrence of CM. The odds ratio of CM increased significantly, as udder morphology was worsened, teat ends were flat and the distance from teat ends to the floor decreased. Hygiene scores of cows were significantly associated with CM prevalence. The ten-point mastitis control program is based on hygiene and includes teat disinfection, antibiotic therapy, and culling of chronically infected cows. Periodically screening protocols for monitoring udder health is another approach for preventing the spreading of mastitis in dairy herds. Positive CMT reaction may be a good indicator for IMI; there was a significant association between the frequency of isolation of major pathogens and the CMT score in milk samples. Developing and following good biosecurity plans takes time and planning, but the cost to the farm enterprise for not having these plans can be considerable.

Keywords: dairy management, clinical mastitis, risk factors .

INTRODUCTION

Mastitis is the most frequent and costly production disease affecting dairy cattle (Berry et al., 2003). 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). Records for the occurrence of clinical mastitis in dairy cows have great importance for monitoring the health status of mammary gland and implementation of programs for control and eradication of this disease.

Incidence and prevalence of clinical mastitis (CM) reported in the literature vary considerably due to differences in the definitions of disease or criteria used for inclusion of the cases (Sargeant et al., 1998; Shpigel et al., 1998). Incidences of CM also vary considerably by country or region where the surveys have been made (Sviland and Waage, 2002).

Mastitis occurs in dairy herds from a complex interaction of host, environment and agent. Generally, the most common risk-factors for CM in dairy herds can be divided into individual cow risk-factors and risk-factors from the environment. Many authors report risk factors for CM associated with farm management, hygienic management, breeding environment, milking technology, cow feeding, season of calving and preventive health management (Nakov et al., 2014). In an individual herd, cow factors are responsible for the difference among cows in having 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 of the first calving, milk leaking, udder edema, milk production, number of milk somatic cells and reproductive disorders (Peeler et al., 2000; Nyman et al., 2007; Valde et al., 2007).

Control of inflammation of the mammary gland and the reduction in their appearance in the herd is based on the number of somatic cells in milk and the incidence of clinical mastitis (IDF, 1997). The measurement used most commonly to detect subclinical mastitis is the somatic cell count (SCC) of milk. Thus, in order to minimize the appearance of mammary inflammation, the main goal should be the number of somatic cells below 200,000/ml of milk, and the incidence of clinical mastitis below 20% (Pyoral, 2003; Schukken et al., 2003). According to national legislation in Republic of North Macedonia (FVA, 2012) milk from uninfected mammary glands contains $\leq 100,000$ somatic cells per milliliter. A milk SCC from 200,000 - 400,000/ml is a clear indication that milk has reduced manufacturing properties, which means is not for consumption. This, an increase in the SCC of milk is a reasonably good indicator of udder inflammation.

MATERIAL 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 Republic of North Macedonia. Selection criteria were set to have a reasonable number of milking cows in target dairy farms ($n \geq 50$) and cows had high milk production (daily milk yield per cow more than 20 kg). Each of these farms differs in the systems and technology of rearing, the milking technology, the size of the herd, hygiene management and health management. The research was divided in four seasons during the year.

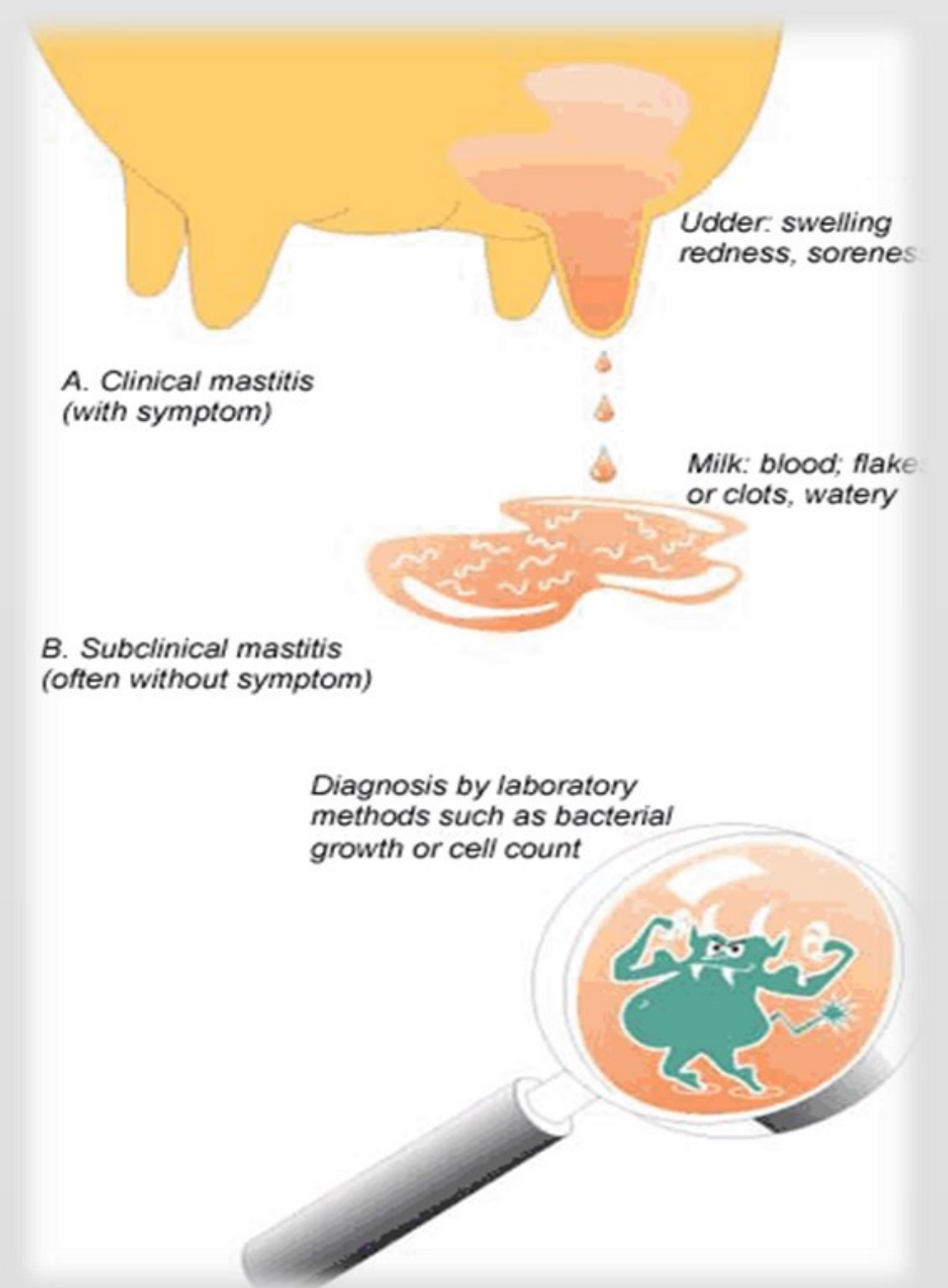
The total number of cow-years at risk was the sum of days in lactation for all cows divided by 365. A cow in lactation was contributing to the sum of cow-years from the day of beginning the trial or the day of parturition within the trial period until the end of trial or day it was drying off or culled. The parity of cows was calculated from the number of consecutive cow's lactation. Ages of observed cows were from the first (1) to the sixth and more lactation (6+).

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 of general health condition of the animal. The screening of udder health status was done on a quarter level using California Mastitis Test (CMT) as a predicted tool for detection of quarters with abnormal milk secretion (Schalm и Noorlander, 1957).

Samples for bacteriological culture from each quarter positive to CMT were collected aseptically in sterile 10 mL tubes, without additives according to the National Mastitis Council (NMC, 2001) and kept at 4°C during transportation. Samples were analysed within 12 hours of collection. Bacterial species were identified according to the standard microbiological procedure using a certificated methodology based on the National Mastitis Council standards (NMC, 2001). Cases of CM were recorded on cow and quarter level. The date of calving was used for estimation of median days in milk (DIM) at occurrence of CM case (DIMCM) by using data from the reproductive board.

The parameters used for calculation the occurrence of clinical mastitis were: the prevalence rate of clinical mastitis per 100 cow-years at risk, prevalence rate of clinical mastitis per 100 lactation/years and lactation incidence risk (LIR). Incidence risk of CM 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, respectively four days antibiotic treatment of infected quarters of mammary gland, another four days when the antibiotics persist in milk and in that period milk was waved aside, and the ninth day when there was not any abnormal change in the milk.

The risk factors that have been followed have categorical and continuous values: classification of udder and teat conformation, measurement of the distance from teat end to floor, the position and conformation of udder quarters.



RESULTS AND DISCUSSION

Table 1. Annual prevalence rate of clinical mastitis per 100 cows in lactation

Farms	Cows with clinical mastitis	Healthy cows	Total number of cows	Prevalence rate per 100 cows
A	40	122	162	24.69%
B	181	176	357	50.70%
C	131	381	512	25.59%
Total	352	679	1031	34.14%

Table 2. Annual prevalence rate of clinical mastitis per 100 lactations

Farms	Lactations with case of clinical mastitis	Lactations without case of clinical mastitis	Total number of lactations	Prevalence rate per 100 lactations
A	41	163	204	20.10%
B	209	198	407	51.35%
C	131	525	656	19.97%
Total	381	886	1267	30.07%

Table 3. Annual prevalence rate of clinical mastitis calculate for cows in different lactations

Farms	Lactation						Total
	1	2	3	4	5	6>	
A	21.43%	13.95%	8.51%	26.47%	31.82%	37.50%	20.10%
B	40.77%	52.58%	59.26%	59.18%	46.67%	62.50%	51.35%
C	12.55%	21.77%	19.23%	30.93%	22.73%	47.06%	19.97%
Total	22.20%	31.01%	33.98%	37.78%	29.63%	46.34%	30.07%

Table 4. Lactation incidence risk for clinical mastitis

Farms	Lactation						Total
	1	2	3	4	5	6>*	
A	23.81%	20.93%	17.02%	32.35%	31.82%	37.50%	25.00%
B	83.08%	100.00%	105.56%	108.16%	66.67%	87.50%	95.58%
C	13.36%	26.53%	19.23%	31.96%	22.73%	47.06%	21.49%
Total	36.04%	50.52%	54.83%	52.78%	33.33%	51.22%	45.86%

CONCLUSION

The control of mastitis in dairy herds depends on the identification and elimination of risk factors associated with the environment, the 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. The fact that bovine mastitis is complex disease is leading to presumption that the differences in incidence risk between farms were resulted from differences in environmental factors and farms management.

This prospective longitudinal study has shown that individual cow factors together with farm management are important in influencing the risk of CM during lactation, and these factors indicate a different susceptibility to CM between animals. 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 to reduce the incidence of mastitis and improve the milk quality.

Table 5. Annual incidence risk of CM, median day in lactation when case was diagnosed and lactation relative risk of CM in observed dairy farms

Farm		Lactation						Total
		1	2	3	4	5	6+	
A	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
	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
B	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
	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
C	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
	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
Total	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
	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	

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

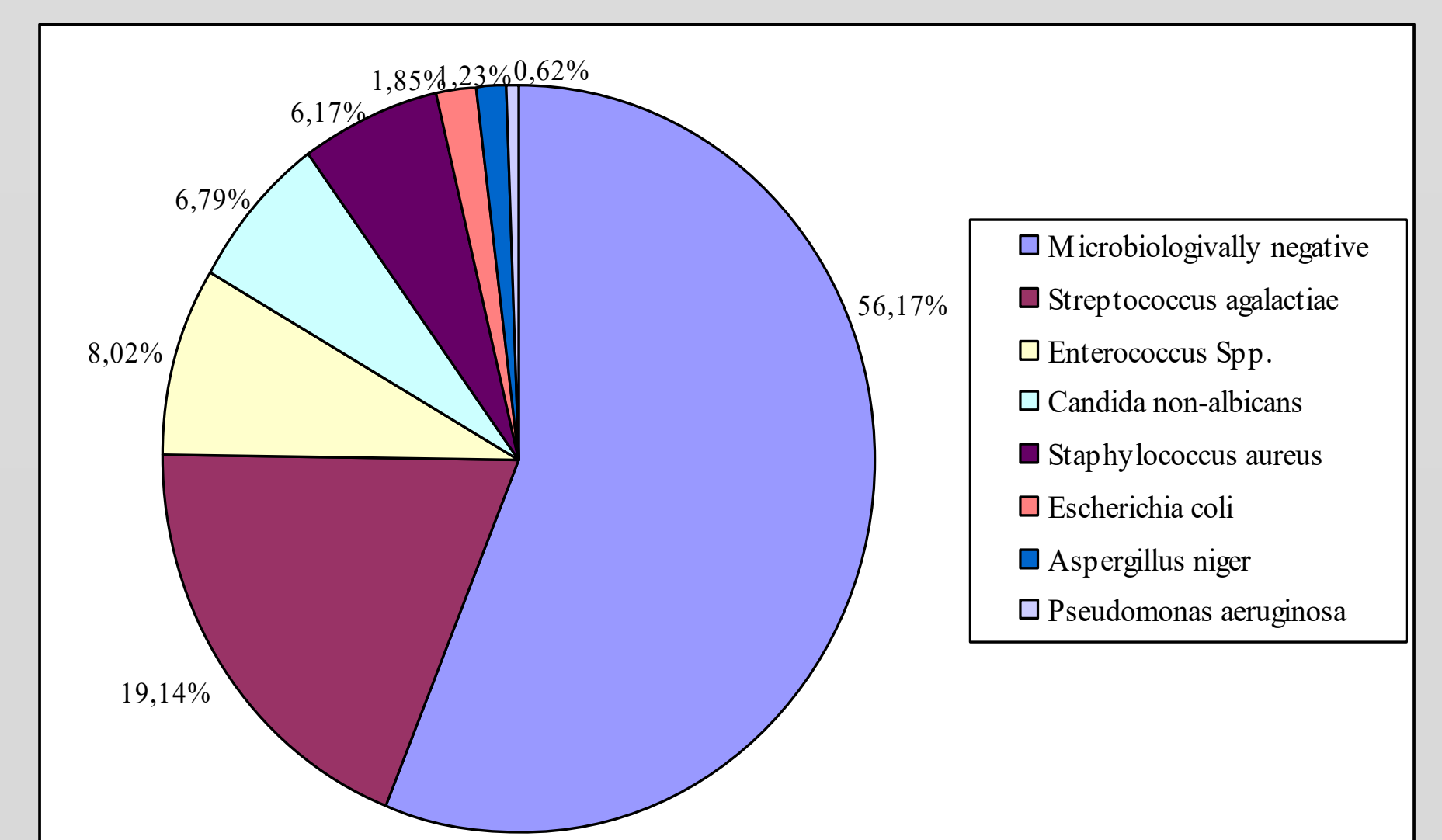
Variable	Level	b	S.E. _b	Wald	df	P-value	odds ratio	95% CI
UF	1	Ref	Ref	24.664	4	P<0.001	1.00	
	2	-0.196	0.150	1.704	1	0.192	0.822	0.612 1.103
	3	-0.718	0.204	12.392	1	P<0.001	0.488	0.327 0.727
	4	0.091	0.190	0.229	1	0.632	1.095	0.754 1.590
	5	0.467	0.253	3.412	1	0.065	1.595	0.972 2.618
TF	1	Ref	Ref	10.115	4	P<0.001	1.00	
	2	0.375	0.088	17.957	1	P<0.001	1.455	1.223 1.730
PF	1	Ref	Ref	10.115	4	0.039	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	1	Ref	Ref	13.458	2	0.01	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	

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

Variable	Level	b	S.E. _b	Wald	df	P-value	odds ratio	95% CI
UR	1	Ref	Ref	39.702	4	P<0.001	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	Ref	Ref	6.779	2	P<0.001	1.00	
	2	0.275	0.074	13.724	1	P<0.001	1.316	1.138 1.522
PR	1	Ref	Ref	9.763	4	0.045	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	1	Ref	Ref	6.779	2	0.034	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	

Table 6. Influence of independent variables on incidence of clinical mastitis

Dependent variable: clinical mastitis			
Source of variations	df	Mean square	F-value
Model	12	538.5836663	1102577.04***
F	2	0.000686378	1.405130301 ^{NS}
L	5	0.000335313	0.685892485 ^{NS}
SY_CM	4	82.93927205	169791.5158***
e	2039	0.000488477	
Total	2051		
R ² = 1,000			



Graph 1. Udder pathogens isolated in milk samples from quarters with positive CMT

The literature data are generally consistent about the reports that with increasing the parity of cow or the number of consecutive lactation, also increase the risk for occurring a case of clinical mastitis.

It is well established that a favorable association exists between mastitis resistance and several udder type traits. Literature data are generally similar regarding genetic correlation between udder depth, udder attachment to cow body, milk production and their association with mastitis incidence (Sorensen et al., 2000; Klein et al., 2005; Ptak et al., 2011). However, must be underlined that clinical mastitis also influences udder and teat morphological characteristics (Klaas et al., 2004).

The udder teats are the body's first line of defence against intramammary infection. The probability of mastitis occurrence varies considerably between different teat shapes, sizes, teat placement and morphology of teat tip (Bardackcioglu et al., 2011).

Some studies reported that decreasing teat-end to floor distance was 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).