



## Original Research Article

### Study of the relationship between certain environment and genetic factors with the different forms of mastitis by cows for milk

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#### A B S T R A C T

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lactation

The study included 395 cows from the Bulgarian Black and White breed, which are breeding in five farms, from two regions in Bulgaria. The cows were at the age of the I-st to III-rd lactation and were daughters of 47 bulls. Rearing is house-bind, with the exception of one, where is applied freely breeding in individual pens. Milking of cows is the respectively with the central pipeline and milking hall - 2x4 seats. The capacity of farms was in the range of 70-80 up to 200 cows and it is with milk production for lactation of 4400 up to 6900kg. With the study aims to determine the connection between the various forms of mastitis with fixed and random factors, as well as with covariate trait - distance to the bottom of the udder from the floor. To achieve the goal are analyzed individual samples milk from each quarter of the udder. At each sampling of the samples is insured material, which is corresponding to the 20 - 30% of the capacity of the farm. The relative share of healthy quarters of the udder are accounted for 66.69 %. With the higher the relative share of the different forms of mastitis to affected quarters is the subclinical mastitis (SCM) - 45.37%, should nonspecific mastitis (NSM) - 25.68; the latent infection (LI) - 24.13 and clinical mastitis (CM) with 4.82 %. The various forms of mastitis is in a different degree of reliable connection with the investigated factors. The latent infection and NSM in connection with only season of the year ( $P < 0.001$ ), while SCM and total mastitis are in connection with only farm, respectively at  $P < 0.001$  and  $P < 0.01$ . It is not established reliable connection with lactation and lactation month. The cow as random factor is not in connection with only NSM, while father of the cow is in connection with only NSM and total mastitis. Only LI and CM are not in related with covariate trait distance of the bottom of the udder from the floor.

#### Introduction

The cows to milk must have many skills, but one of the most important is to deal

with a number of diseases, mostly with the mastitis.

The subclinical and clinical mastitis are one of the most significant problems in dairy cattle breeding, which no doubt lead to greater economic losses, resulting in reducing the quantity and quality of raw milk, and subsequently, on the quality of milk products. On the other hand is not small per cent of premature exit of cows from the main herd, exactly for this reason.

According to Tolsma (2007), at least 10 are the main factors (such as genetic and non-genetic), which are associated with the disease of mastitis. In two consecutive studies Nash et al. (2002, 2003) examined the connection between the age of cows and the clinical cases of mastitis. The I-st lactation were in the range of 11 to 55%, and for II-nd lactation of 12 to 46%. At the average milk traits of 6608 kg, Rupp and Boichard (1999) have found 12.6% CM when cows of I-st lactation. Querengässer et al. (2002), Rajala-Schultz et al. (2004), Kocak (2006), Reksen et al. (2007) also considered that the incidents of mastitis in connection with the age of cows.

The season of the year is a factor that has an influence in different degree. According to Wendt and Jautze (1979), the frequency of the disease of mastitis is on the rise in the summer, as this is linked with an increase in the ambient air temperature.

In Bulgaria, Tsolov (1989) identify the different forms of mastitis, as follows: subclinical mastitis (SCM); latent infection (LI); nonspecific mastitis (NSM); clinical mastitis (CM) and healthy mammary gland. In Member States of EU and USA, subclinical mastitis is defined in  $SCC \geq 250\ 000/ml$  and the presence of  $>500\ cfu/ml$  of

pathogenic micro-organisms (National Mastitis Council, 1999). The different topography of quartering of the udder, especially front: Rear is in connection with health status of the udder. According to Tsonev et al. (1975), at 5-they studied breeds in Bulgaria sensitivity to subclinical mastitis of the hind quarters is nearly two times greater than the front.

Lam et al. (1997) examined effects of interruption in the disinfection of teats after milking the cows. In conclusion the authors indicate that the immediate disinfection can reduce incidents of mastitis caused by *E. coli*.

According to Ziggers (2004), the therapy at the dry cows may reduce the risk of a new infection of the udder for more of 30%. The therapy with antibiotics at the end of lactation is the most effective method for the elimination of the existing infection. The aim of this study is to determine the connection between the various forms of mastitis with fixed (a farm; lactation; lactation month and season of the year) and random (father of the cow and cow) factors, as well as with covariate trait - distance to the bottom of the udder from the floor.

## Materials and Methods

In this study were included 395 cows from the Bulgarian Black and White breed, which are breeding in five farms, from two regions in Bulgaria and they were daughters of 47 bulls. They were of age: I<sup>-st</sup> lactation (215 number); II<sup>-nd</sup> (102) and III<sup>-rd</sup> lactation (78 number). Rearing the cows in farms was house-bind, with the exception of one, where is applied freely breeding in individual pens. The milking of the cows is the respectively with the central pipeline and milking hall - 2x4 seats. The capacity of

farms was in the range of 70-80 up to 200 cows and they are with milk production for lactation of 4400 up to 6900kg.

According to lactation month, the distribution of cows is the following: 1 - 2 month- 81 number; 3 - 4 month - 105; 5 - 6 month - 111 and for 7-8 month - 98 number. To achieve the goal are taken into individual samples milk from each quarter of the udder, and their total number corresponding to 1555. In exploratory period are found 25 number quarters, which were not working. At each sampling of the samples we have provided material, who met the 20 - 30% of the capacity of the farm.

Collecting and the transport of the aseptic milk samples for bacteriological examination were monitored according to the instructions described by the National Mastitis Council - NMC (1990).

The health status of the milk gland is determined according to the requirements of the International Federation of Dairy (1981, 1987), according to which are: subclinical mastitis (SCM), latent infection (LI), nonspecific mastitis (NSM) and clinical mastitis (CM). According to the new standards, subclinical mastitis definition in number of somatic cells  $\geq 250\ 000/\text{ml}$  and the presence of mastitis pathogens  $\geq 500\ \text{cfu}/\text{ml}$  of the milk.

The isolation and identification of micro-organisms we used accredited methodologies based on NMC [13]. The quarters was take for bacteriological positive with growth of  $\geq 500\ \text{cfu}/\text{ml}$  of the milk, and samples with  $>2$  bacterial species such as contaminated. In the case of growth of two different species, according to the chief pathogen:

*Staphylococcus aureus* >*Streptococcus uberis* / *Streptococcus spp* >CNS >*Corynebacterium bovis*.

The study of the relationship between factors and the different forms of mastitis we used 347 cows, such as: At number 144 number are taken into a single sample. The 116 number - to respond to two, but three consecutive samples are supplied by 87 cows. The cows were daughters of 46 bulls.

The investigated factors that participate in linear models we view them as fixed, random and covariate and distribution in classes is as follows:

- Farm - in 5 classes (farm A; H; S; I; M;
- Lactation - in 3 class (I-st; II-nd, and III-rd lactation);
- Lactation month - in 4 classes (1-2); 3-4; 5 - 6 and 7-8- lactating animals month) and
- Season of the year - in 3 class [Autumn (IX-XI month from 2007 year); Winter (XII-II) and spring (III-V month from 2008 year)];
- Distance to the bottom of the udder from the floor - in the 5- class: Up to 50 cm; 50.1-55.0 cm; 55.1 - 60.0 cm; 60.1 - 65.0 cm and above 65.1 cm.

For the study of the relationship between the observed factors, and treats is used the programming package Statistica 6, algorithm modes can be selected component - presented as fixed and random effects, the effects of covariate treat - distance for the bottom of the udder from the floor. Mixed linear model is the following:

$$Y_{ijklmn} = \mu + A_i + L_j + LM_k + H_l + S_e + C_m + K_n + e_{ijklmn}, \text{ where:}$$

$Y_{ijklm}$  - is spelling variable (studies indicate);

$\mu$  - the average for the general population;

$A_i$  -  $i$  - the effect of the fixed factor farm;

$L_j$  -  $j$  - the effect of the fixed factor lactation;

$LM_k$  -  $k$  - the effect of lactation month;

$H_l$  -  $l$  - the effect of season of the year;

$S_e$  -  $e$  - the effect of random factor father of cow;

$C_m$  -  $m$  - the effect of random factor cow;

$K_n$  -  $n$  - the effect of covariate indicate remoteness of the bottom of the udder from the floor and

$e_{ijklm}$  - impact of several random effects.

Multiple comparisons between groups were calculated with the aid of LSD (Post host) - also from the aforementioned program package.

## Results and Discussion

In Table 1 are presented data from healthy and infected quarters of the udder of the different forms of mastitis. Healthy quarters accounted for 66.69%, and according to place location - Front (right and left) and Rear are in borders, respectively, from 66.50 to 71.10% and from 64.71 to 65.22%.

The LI is an average 8.04%, with deviation according to the place of location in quartering method - from 7.42 to 9.46%. The share of NSM from all quarters is 8.55%, as of the individual titles is from 7.16 (Front left) to 9.72% (the Right quarters).

In the case of SCM we have seen that over 16% of the hind quarters are infected by this mastitis, and in the front is about 14%. Clinical mastitis is set at 1.61% of the surveyed quarters, and the quarters of the udder the deviation is 1.02 (Front left) to 2.05% (Rear right). For the entire mammary glands, with the highest share

of the different forms of mastitis is SCM (15.11%) are NSM (8.55%), LI (8.04%) and CM (1.61%).

The study of Barkema et al. (1997) is in accordance with our data. The authors indicate that infection in the fore quarters is less, than in the rear. Such a trend is retained and the species of buffalo. Chavoshi and Husaini (2012) have been studying quartering method of the udder in buffalo and have found that by SCM were infected 73% of the hind quarters, while in the front the title corresponding to 27%. In this study, but in the same form of mastitis (SCM) this ratio is 32.20:27.80% and indicates that the hind quarters are infected in more by around 5%, to the front.

In Poland, Hameed et al. (2006) as well as the us ones distributed mastitis in these four forms, but they receive a higher percentage of SCM and LI, respectively 25.35 and 26.19%. Likely this is due to the fact that the processing of the information is based on number of cows. This is due to its lowest rate the relative share of healthy animals - 39.19%.

Various forms of mastitis, as the relative share of affected quarters is the following - table 1: 45.37%; 25.68%; 24.13% and 4.82%, respectively, for SCM, NSM, LI and CM. These data show the significant burden of SCM to general illness of mastitis. Indicative are therefore and the maximum number of infected quarters of the udder. For LI and CM this corresponds to the 2-, for NSM of 3- and for SCM of 4- quarters.

Included in the model 6-factor, only the season proved highly faithful influence ( $P < 0,001$ ) on this form of mastitis (Table 2).

Cow as random factor is in connection with an average degree of confidence. The utilization the values of the variance must detect that leading is the influence of the season of the year, followed by farm and the lactation month.

For the model, the LS- mean is 0.3 quarters infected by LI (Table 7). In the winter and the spring is found higher values of such valid for model, respectively +0.1 and +0.2 quarters. Higher temperatures in the spring create and more favorable conditions for the development of pathogenic microorganisms, which are the basis for the development of this form of mastitis.

Nonspecific mastitis as a part of hidden mastitis at the cows is significantly influenced by the fixed factor season of the year ( $P < 0.001$ ) - Table 3. Father of the cow is in connection with this form of mastitis with an average degree of confidence. Such is the relationship and with covariate treat, which has a leading role - utilization the values of the variance, follows the season of the year, and farm.

This form of mastitis is closely linked with increases SCC and the leading role of the remoteness of the bottom of the udder from the floor is more than logical. Cows that have had mammary glands at the distance nearer to the floor and who had pendulous and terraced form of the udder are created the conditions for greater variability, to animals with more shallower mammary glands and such situated forward on abdomen.

For the model, LS-mean is 0.3 c number quarters (Table 7), which are disease during milking of the animals. Higher values than those for model are

established in the autumn and winter, respectively LS-estimation (LSE) is +0.2 and +0.1 quarters. Most likely this is related to the larger dirt particles of the premises and the rear part of the body, but most are already in the mammary glands. An analysis of the results in the spring indicates that the disease of this form is at least as compared to model (with negative LSE = -0.2 quarters), so with the other two seasons. The difference is reliable at  $P < 0.01$  (winter) and  $P < 0,001$  (autumn).

The factors farm is the only fixed-factor, which is in the high reliable connection with SCM. Cow as random factor is at the same level of reliable connection. Based on the values of the variance we have seen, the farm is the leading factor for this form of mastitis, as the variance is at times more than in other factors, Table 4. This means that a optimum compliance of prevention activities related to breeding and milking the cows, can contribute to reducing the disease of SCM, but as a whole of mastitis. We accept such a hypothesis, as above with that about 45% of the mastitis is in connection with this form of the disease.

The lactation as a fixed factor is arrange to second place from the point of the value of the variance. Connection is non-significant with the participation of cow as random factor, while participation only the fixed factors of the: A farm; Lactation; Lactation month and season of the year, the age of cows presented as lactation is at a high degree of reliable connection with this form of mastitis. In this sense our results are in accordance with those obtained from Busato et al. (2000). The authors indicate that with increasing age of cows the risk of mastitis is on the rise.

The average incidence of quartering method of the udder from this form of mastitis is 0.6 - table 7. Depending on the farm, a greater infection is evidenced on the farm S and M, respectively the LS-estimation are a positive sign (+0.7 and +0.2), and with significantly more healthy udders were cows from the farm, and, and, where LS-evaluation, it is respectively negative, -0.5 and -0.4 quarters.

Data related to the age of the animals show that LS-evaluation is positive for the average model, but only for cows of III<sup>rd</sup> lactation (+0.2 quarters). For those of I<sup>st</sup> and II<sup>nd</sup> lactation is negative (-0.1). Such a study is conducted by Ayano et al. (2013). The authors analyze the data based on age and period of lactation, but the number of cows, and not the number of quarters. Probably this is the reason for higher rates of this disease, which was 37.30% in animals to 6- years and 46.42% of those over 6- years of age.

Data presented in table 5 show that only cow as random factor is in connection with this form of the mastitis. This is sufficiently meaningful, that no other factors are not created conditions for disease of the udder from this form of mastitis, unless the cow.

As for the gravity of the involved factors - submitted by the values of the variance should express that lactation has the leading role, should farm. Probably this is the reason these two factors are reliable associated with clinical mastitis, but when participate in model only as the fixed factors.

The average incidence of quartering method of the teats of this form of mastitis is 0.06 number - Table 7. It is

worth consideration that during the winter period in any farm is not established such a disease.

In the general sickness of mastitis - without taking account of its various forms found that of the fixed factors, only that the farm is in reliable connection with mastitis - at  $P < 0.01$  (Table 6). Random factors (father and cow) are also in reliable connection, respectively at  $P < 0.05$  and  $P < 0.01$ , as well as covariate treat ( $P < 0.05$ ).

It deserves attention random factor cow, because only with NSM is not reliable connection. In practice each one of them is different in pedigree and morphological characteristic of the udder, as remoteness from the floor, the shape of the udder and the teats, elasticity of the sphincter and i. e. According to us, it is sufficiently meaningful to point at here that is more than necessary to take account of the different forms of mastitis.

A typical example is that, in this disease the season of the year is non-significant connected until, with LI and NSM (Table 2 and 3) is at a high level of connection and shows that, during certain seasons should focus its efforts toward prevention, to reduce the relative share.

On the other hand found it is inappropriate that lactation and lactation month are not reliably linked as fixed factors, as well as to the various forms of mastitis, and in the general sickness of mastitis. In other words, it also means that the disease will affect animals of different ages at the same time and in different periods of lactation.

**Table.1** Relative part in the differences types of the mastitis in the individual quarters in the udder

Health quarters and that infected with differences types of the mastitis	Q u a r t e r s				Total	
	FR	RR	FL	RL	N	%
	%	%	%	%		
Health	66.50	64.71	71.10	65.22	1037	66.69
LI	7.67	7.42	7.42	9.46	125	8.04
NSM	9.72	9.72	7.16	7.42	133	8.55
SCM	14.58	16.10	13.30	16.10	235	15.11
CM	1.53	2.05	1.02	1.79	25	1.61
Total	100.0	100.0	100.0	100.0	1555	100.00

*Spread of the various forms of mastitis - general and depending on place available of the quarter of the udder.*

**Table.2** Relationship between the factors with Latent infection in to the udder

Factors	Effects	df	MS	F
Distance between bottom of the udder and the floor	Fixed	1	0,678	2,21
1. Farm	Fixed	4	1,096	6,08
2. Lactation	Fixed	2	0,210	0,64
3. Lactation month	Fixed	3	0,348	1,17
4. Season of the year	Fixed	2	2,255	7,92***
5. Father of the cow	Random	44	0,319	0,78
6. Cow	Random	181	0,383	1,62**

*Connection between fixed and random factors and covariate indicate remoteness of the bottom of the udder of the floor with the latent infection (LI) in the udder.*

**Table.3** Relationship between the factors with Nonspecific mastitis in to the udder

Factors	Effects	df	MS	F
Distance between bottom of the udder and the floor	Fixed	1	4,108	6,17**
1. Farm	Fixed	4	2,576	2,21
2. Lactation	Fixed	2	0,090	0,14
3. Lactation month	Fixed	3	0,223	0,56
4. Season of the year	Fixed	2	3,490	8,92***
5. Father of the cow	Random	44	0,676	1,74**
6. Cow	Random	181	0,382	1,11

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001

*Connection between fixed and random factors and the covariate treat remoteness of the bottom of the udder from the floor with Nonspecific mastitis in the udder*

**Table.4** Relationship between the factors with Subclinical mastitis in to the udder

<b>Factors</b>	<b>Effects</b>	<b>df</b>	<b>MS</b>	<b>F</b>
Distance between bottom of the udder and the floor	Fixed	1	2,581	2,93
1. Farm	Fixed	4	17,350	15,91** *
2. Lactation	Fixed	2	1,337	1,44
3. Lactation month	Fixed	3	0,205	0,36
4. Season of the year	Fixed	2	0,609	1,14
5. Father of the cow	Random	44	0,922	1,10
6. Cow	Random	181	0,774	2,15***

*Connection between fixed and random factors and the covariate treat remoteness of the bottom of the udder from the floor with sub-clinical mastitis in the udder.*

**Table.5** Relationship between the factors with Clinical mastitis in to the udder

<b>Factors</b>	<b>Effects</b>	<b>df</b>	<b>MS</b>	<b>F</b>
Distance between bottom of the udder and the floor	Fixed	1	0,052	0,62
1. Farm	Fixed	4	0,110	1,10
2. Lactation	Fixed	2	0,212	2,36
3. Lactation month	Fixed	3	0,070	1,32
4. Season of the year	Fixed	2	0,062	1,27
5. Father of the cow	Random	44	0,089	1,06
6. Cow	Random	181	0,076	2,60***

*Connection between fixed and random factors and covariate treat remoteness of the bottom of the udder of the floor with clinical mastitis in the udder.*

**Table.6** Relationship between the factors with the total disease in the udder of the mastitis

<b>Factors</b>	<b>Effects</b>	<b>df</b>	<b>MS</b>	<b>F</b>
Distance between bottom of the udder and the floor	Fixed	1	8,963	4,69*
1. Farm	Fixed	4	21,797	6,85**
2. Lactation	Fixed	2	0,059	0,03
3. Lactation month	Fixed	3	0,135	0,13
4. Season of the year	Fixed	2	2,043	2,00
5. Father of the cow	Random	44	1,964	1,55*
6. Cow	Random	181	1,196	1,54**



**Table.7** LS-mean (LSM) and LS-estimation (LSE) of the significant connects the factors in to the different type of the mastitis and total mastitis

Factors	n	T y p e of the m a s t i t i s						Total mastitis	
		LI		NSM		SCM		LS M	LS E
		LS M	LSE	LS M	LSE	LS M	LSE		
Average for the model	<b>347</b>	<b>0.3</b>	-	<b>0.3</b>	-	<b>0.6</b>	-	<b>1.3</b>	-
Farm: S	63	-	-	-	-	1.4	+0.8	2.2	+0.9
M	95	-	-	-	-	0.7	+0.1	1.5	+0.2
H	74	-	-	-	-	0.1	-0.5	0.8	-0.5
I	29	-	-	-	-	0.2	-0.4	1.1	-0.2
A	86	-	-	-	-	0.5	-0.1	1.0	-0.3
Season: Autumn	144	0.2	-0.1	0.5	+0.2	-	-	-	-
Winter	76	0.4	+0.1	0.4	+0.1	-	-	-	-
Spring	127	0.5	+0.2	0.1	-0.2	-	-	-	-
Distance between bottom of the udder and the floor, cm: by 50	42	-	-	0.6	+0.3	0.8	+0.2	1.8	+0.5
50.1 - 55	73	-	-	0.4	+0.1	0.7	+0.1	1.5	+0.2
55.1 - 60	138	-	-	0.3	±0.0	0.5	-0.1	1.2	-0.1
60.1 - 65	76	-	-	0.2	-0.1	0.6	±0.0	1.3	±0.0
above 65.1	18	-	-	0.3	±0.0	0.3	-0.3	1.1	-0.2

In confirmation of our results is conclusion of Avoncroft (2004), which indicates that management decisions are relevant to the greatest extent for the dissemination of the mastitis (65%) and adding and the feeding of cows by 20%, it should emphasize that are very large reserves, which may contribute to longer retention of the animals in the herd and the production of better quality milk.

The average values for model show that

1.3 of quartering method of the udder are infected from mastitis, a higher result from this model are reported on the farm S and M, respectively, with +0.9 and +0.2 - Table 7.

This in absolute values corresponds to 2.2, and 1.5 quarters - infected of mastitis. High results in these farms are due to the illness of quartering method of the udder, but most of SCM and NSM. With this fact it may be suggested that in them,

there are many outstanding organizational issues and are not carried out maintenance events.

For other farms is in the range from 0.8 to 1.10 number quarters. To a considerable extent the difference ( $P < 0.001$ ) between the first two farms and the other is due to the fact that the latter are complied with and it largely basic requirements in the cultivation, milking the cows, prevention during lactation, at the drying cow and during dry period.

It deserves attention the connection between the remoteness of the bottom of the udder from the floor and the prevalence of mastitis. Cows with the udder, who stood as close to the ground (up to 55 cm) had a greater number of infected quarters, as well as to the average on the model, and with those having a shallower such. Reliable difference (at  $p < 0.05$  to  $P < 0.01$ ) found between animals with mammary glands, whose bottom is up to 50 cm from the ground and those with over 55.1 cm.

The study factors - farm, season of the year, father of cow, cow and covariate indicate remoteness of the bottom of the udder from the floor is in a different degree of reliable connection with the individual forms of mastitis, as well as with the total illness of mastitis.

The Latent infection is in connection with only a fixed factor season of the year and with random factor cow. The Nonspecific mastitis is in reliable connection (at  $P < 0.01$  -  $P < 0.001$ ) with covariate indicate remoteness of the bottom of the udder from the floor, the season of the year, and the father of cow. On a low to a high degree of reliable connection is Sub-clinical mastitis with distance to the

bottom of the udder from the floor, farm, and cow. Clinical mastitis is in reliable connection with only random factor cow.

Only lactation, lactation month and the season of the year are not in connection with the general disease of mastitis. The first two fixed factors are not in connection with various forms of mastitis. The ill ones quarters of the udder, the highest the relative share of the different forms of mastitis is the Sub-clinical mastitis - 45.37%, should Nonspecific mastitis - 25.68%; The Latent infection - 24.13% and Clinical mastitis with 4.82%. The maximum number of infected quarters according to form of mastitis has been in the range 2 (of the Latent infection and Clinical mastitis) to 4-quarters of Subclinical mastitis.

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