Kocatepe Veterinary Journal

Kocatepe Vet J (2016) 9(3): 185-193
DOI: 10.5578/kvj.27875
Submittion: 14.06.2016
Accepted: 26.07.2016

RESEARCH ARTICLE

Effect of Some Risk Factors on Subclinical Mastitis in Dairy Cows#

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This study was financially supported by Afyon Kocatepe University Scientific Research Commission (11.SAG.BIL.05) under thesis project.

ABSTRACT

The present study was performed to investigate the effect of some risk factors on subclinical mastitis in dairy cows. California Mastitis Test (CMT) was performed on 774 mammary quarters of 195 Holstein Friesian, Swiss Brown and Simmental cows and at least one CMT positive 125 milk samples of 100 cows were included into the study. Milk samples were evaluated microbiologically and antibiotic sensitivity test was carried out. CMT positive cows rate was 51.28% and 63% of these animals showed positive microbiological growth. CMT positive samples percentage found 16.14% in all mammary quarters (n:774) and 60.80% of milk samples collected from CMT positive mammary quarters showed microbiological growth. *E.coli, Candida* spp.*, S.uberis,* Coagulase Negative Staphylococcus*, S.aureus, Proteus* spp.*, and Bacillus* spp. were isolated as 28.9, 24.21, 19.53, 19.53, 3.9, 2.34 and 1.56%, respectively. According to farm localization, CMT positive cows did not show significant difference between lactation number/period and age. Moreover, farm localization, age, lactation number/period, breed and localization of mammary quarters did not affect CMT scores and microbiological results. Microbiologically positive samples showed that microorganisms were sensitive against amoxicillin+clavulanic acid and oxytetracycline, whereas those were resistant against gentamycin, ceftiofur, enrofloxacin and cefquinome. In conclusion, it is thought to be that age, breed, lactation number and lactation period of cows are not effective factors causing subclinical mastitis as much as inadequate hygiene, improper milking system and mastitis control programs. Furthermore, it is strongly suggested that ampiric antibiotic therapy can be an important risk factor for contributing antibiotic resistance.

Key words: Age, Antibiotic Sensitivity Test, Dairy Cow, Lactation Number, Lactation Period, Mammary Quarter's Position, Risk Factors, Subclinical Mastitis

Sütçü İneklerde Bazı Risk Fatörlerinin Subklinik Mastitis Üzerine Etkileri

ÖZ

Sunulan çalışma, süt inekçiliği işletmelerinde subklinik mastitis oluşumuna etki eden bazı risk faktörlerini araştırmak için yürütüldü. Yüzdoksanbeş Holştayn, Esmer ve Simental ırkı sütçü ineklerin 774 meme lobuna Kaliforniya Mastitis Test (CMT) uygulandı ve en az bir meme lobu CMT pozitif reaksiyon veren 100 ineğin 125 meme lobundan mikrobiyolojik yoklamalar ve antibiyotik duyarlılık testleri için süt örnekleri alındı. CMT pozitif inek oranı %51.28 olarak belirlenirken, bu hayvanların %63'ünün sütünde üreme gözlendi. Meme loblarının (n:774) %16,14'ünde CMT pozitif reaksiyon gözlenirken bunların %60,80'inde üreme gözlendi. Mikrobiyolojik yoklamalarda *E.coli* %28.9, *Candida* spp. %24.21, *S.uberis* ve Koagulaz Negatif Stafilokoklar %19.53, *S.aureus* %3.9, *Proteus* spp. %2.34 ve *Bacillus* spp. %1.56 oranlarında gözlendi. CMT pozitif olan hayvanların yerleşim yerlerine göre laktasyon sayısı/dönemi ve yaş ortalamaları arasında istatistiki fark belirlenemedi. İneklerin yerleşim yerinin, yaşlarının, laktasyon sayılarının/dönemlerinin, ırk özelliklerinin ve numunelerin alındığı meme lobu lokalizasyonunun CMT skorlarına ve mikrobiyolojik sonuçlara etkilerinin olmadığı gözlendi. Çalışmada üreyen bakterilerin amoksisilin+klavulonik asit ve oksitetrasiklin gibi antibiyotiklere duyarlı oldukları, gentamisin, seftiofur, enrofloksasin ve sefkuinom'a dirençli oldukları tespit edildi. Sonuç olarak ineklerin yaşı, ırkı, laktasyon sayısı ve laktasyon dönemi gibi faktörlerin subklinik mastitis oluşumunda, yetersiz hijyen, uygun olmayan sağım sistemi ve mastitis kontrol programları kadar etkili olmadıkları düşünülmektir. Ayrıca bilinçsizce kullanılan antibiyotik tedavilerinin bazı antibiyotiklerin bakterilere karşı dirençli hale gelmesinde önemli bir risk faktörü olduğu ileri sürülebilir.

Anahtar Kelimeler: Antibiyotik Duyarlılık Testi, Laktasyon Dönemi, Laktasyon Sayısı, Meme Lobu Pozisyonu, Risk Faktörleri, Subklinik Mastitis, Sütçü İnek, Yaş

To cite this article: **Koçyiğit R, Yılmaz O, Özenç E, Uçar M.** Effect of Some Risk Factors on Subclinical Mastitis in Dairy Cows. *Kocatepe Vet J. 2016; 9(3):185-193.*

INTRODUCTION

Mastitis is the inflammatory reaction of mammary gland and causes economic loss in dairy cattle. Breed, age, milk yield, lactation period and number, milking, season, feeding and housing conditions play key roles in aetiology of mastitis (Baştan 2010). Bacteria (*S.aureus, S.agalactiae, Mycoplasma bovis*, *Corynebacterium bovis,* environmental *streptococci* and c*oliforms*) and their toxins (Murinda et al. 2002), yeast and fungi are responsible from the infection (Stanojevic and Krnjajic, 2004).

Clinical mastitis is characterized by visible inflammatory changes of udder and milk. Subclinical mastitis, the most common form of mastitis, does not cause any visible changes in udder and milk however, it is diagnosed by some clinical and chemical tests as well as bacteriology and the presence of increasing somatic cell count (SCC) (Baştan 2010).

The healthy milk consists of 200.000 somatic cell per ml (Baştan 2010). Increased SCC is a criteria for diagnosis of subclinical mastitis. California Mastitis Test (CMT) is a simple cow-side indicator of the somatic cell count and pH of milk. It provides a useful technique for detecting subclinical mastitis. It operates by disrupting the cell membrane of any cells present in the milk sample, allowing the DNA in those cells to react with the test reagent, forming a gel (Schalm et al. 1971; Philpot and Nickerson 1991). The isolation, identification and antibiotic sensitivity tests should be performed following the detection of higher SCC in milk samples (Kuyucuoğlu and Uçar 2001).

Subclinical mastitis depends on many risk factors such as location of the farms, milking procedures and conditions of cows. The study was performed to reveal the effect of location of farms and condition of animals (age, lactation number, lactation period, breed and mammary quarters) on subclinical mastitis diagnosed by CMT and bacteriology in dairy farms in Mudurnu region of Bolu, Turkey.

MATERIALS and METHODS

The study was performed in 195 dairy cows (Holstein Friesian, Brown Swiss and Simmental) housed in 32 farms located in five settlements (named as A, B, C, D and E) in Mudurnu region of Bolu. The CMT was applied to total of 774 mammary quarters following the inspection and palpation of each udder. Accordingly, at least one mammary quarter of 100 cows displaying CMT positive reaction was evaluated. The information of age, lactation number and lactation period (month) of the animals was recorded. Moreover, CMT results were evaluated as CMT +1, +2 and +3; mammary quarters (n: 125) were recorded as right front, right rear, left front and left rear. Sterile milk samples collected from CMT positive mammary quarters were transported to laboratory of Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, at + 4 ºC.

Isolation and identification of bacteria was performed in a routine manner described by Koneman et al. (1992). Kirby-Bauer Disc Diffusion method was used to apply antibiotic sensitivity tests. In order to determine susceptibility, the discs of amoxycillin+clavulonic acid 30µg, enrofloxacin 5µg, gentamycin 10µg, oxytetracyclin 30µg, penicillin 10µg, ceftiofur 30µg, cefquinome10µg were used and evaluated according to the primary, secondary, and tertiary zone diameters.

The statistical analysis of data obtained throughout the study was performed by using SPSS 16.0 program. Chi square test was used to determine the difference between the locations of farms. The differences between the average value of age, lactation number and lactation period as compared to locations of farms were tested by one way variance analysis (ANOVA). Tukey and Duncan tests were used to determine the significance between and in groups. The effect of location of farms, age, lactation number, lactation period, breed and udder on CMT scores (+1, +2, +3) and bacteriology (+: 1, -: 0) was evaluated by univariate variance analysis (General linear model). The relationship between CMT scores and the presence of bacteria was evaluated by Pearson correlation analysis

RESULTS

The distribution of breed and number of 100 cows due to the locations is given in Table 1. According to the locations, there were no statistical differences between CMT positive cows and also between bacteria isolated cows (Table 2). Moreover we did not find any statistical difference between CMT positive mammary quarters and also between bacteria isolated milk samples (Table 2). However, CMT scores and percentage of bacteria isolated from those showed no significant difference as compared to location of the farms (Table 3). Furthermore, the distribution rates of isolation of bacteria in those locations (Table 4) and cows having CMT positive scores and distribution of identified bacteria as compared to udder(s) did not display significant difference (Table 5).

There was no significant difference between age, location, lactation number and lactation period of cows having CMT positive scores (Table 6). Besides, it was observed that location of farms, age, lactation number, lactation period, breed and milk samples collected from udder(s) did not affect the CMT scores and the results of bacteriology (Table 7). The antibiotic susceptibility test showed that the all isolated bacteria were resistant against gentamycin, ceftiofur, enrofloxacin and cefquinome (Table 8).

DISCUSSION

In the present study which subclinical mastitis was firstly researched in Bolu province, the percentage of cows showed CMT positive score(s) was 51.28 % and isolation and identification of bacteria was achieved from 63% of those cows. Ergün et al. (2004) reported that the rate of CMT positive cows was 71.8% in family-type farms in Hatay province, Turkey. The reason of highly detected CMT positive cows may be due to absence of mastitis control programs such as pre and post dipping in family farms as it was suggested in our study (Uçar et al. 1997). In contrary, Alaçam et al. (1986) stated the lower (14.11 %) rates of CMT positive cows. In the present study, the rates of CMT positive cows in different farms display no statistical difference (Table 2). These results showed that rate of CMT positive cows might be high or low, without statistical differences, related to location of farms as well as implementation of mastitis control programs.

It was detected that 16.14% of totally examined 774 mammary quarters showed CMT positive scores. Moreover, bacteria were isolated from 60.80% of those mammary quarters (Table 2). Ergün et al. (2004) reported that they had found 40.90% CMT positive mammary quarters from 640 evaluated mammary quarters. Additionally, aerobic bacteria were isolated from 76.30% of CMT positive mammary quarters. In addition to this, Özenç et al. (2008) stated that bacterial growth was detected only 30.10% CMT>0 scores. The reason of observing no isolation of bacteria from all CMT positive mammary quarters in that reports and our study (Table 2) might be related to agent of infection, some environmental factors and healing process or slightly dated of infection. Moreover, it is suggested that viral infection or aseptic mastitis may also be associated with lack of isolation (Uçar 1999).

The rate of +1, +2 and +3 CMT positive scores was 79.20 %, 16.00 % and 4.80 %, respectively (Table 3). Baştan et al. (1997) reported in their study that +1, +2 and +3 CMT scores were 21.18 %, 30.60 % and 20.20 %, respectively. In our study, we found that the incidence rate of CMT positive cows decreased, when CMT scores increased. Additionally, the rate of isolation of bacteria in +1, +2 and +3 scores were 57.58 %, 85.00 % and 66.67 %, respectively (Table 3). It was observed that unless the rate of isolation of bacteria did not show any significant difference, the rate of bacterial isolations were higher for +2 and +3 CMT scores. This might be related to mild form nature of CMT +1 subclinical mastitis or healing process of any infection. Moreover, it is suggested that milking hygiene and personnel, optimization and management of milking may play role in increasing CMT scores (Çoban and Tüzemen 2007).

In the present study, the bacteriology of cows having positive CMT scores revealed that the isolation rate of *E.coli*, *Candida* spp., *S.uberis* and Coagulase Negative Staphylococcus (CNS), *S.aureus*, *Proteus* spp. and *Bacillus* spp. was 28.9 %, 24.21 %, 19.53 %, 3.9 %, 2.34 % and 1.56 %, respectively (Table 4). Ergün et al. (2004) reported that CNS (%42.60), *S.aureus* (25.20 %), *S.uberis* (11.30 %), *S.agalactiae* (6.5 %), *S.dysgalactiae* (6.50 %), *Bacillus* spp. (2.30 %), *P.auriginosa* (1.70), *S.faecalis* (0.90 %) and *E.coli* (1.70 %) were isolated and identified. Rişvanlı and Kalkan (2002) stated the isolation rates for *S.aureus*, *S.epidermidis*, *S.enteridis*, *Yeast, Streptococcus* spp., *Bacillus* spp., and *Pseudomanas* spp. as 67.17 %, 20.61 %, 4.24 %, 4.85 %, 1.81 %, 0.61 % and 0.61 %, respectively. Similar microorganisms were isolated in our study however, the rate of isolation of them showed differences. The isolation of wide range and number of bacteria in farms might be related to mastitis control programs, milking management, transfer of new cows into the herd and mostly environmental factors. In the present study, it was seen that *E.coli, S.uberis,* CNS and *Candida* spp. were the most dominant microorganisms causing infection, while *S.aureus, Proteus* spp. and *Bacillus* spp. were not isolated in some of the farms (Table 4).

The present study demonstrated that the average age, lactation number and lactation period of cows located in different farms did not show any significant difference between those parameters and location of farms (Table 6). Therefore, it is concluded that those factors might be similarly effective on occurrence of mastitis. However, it was found that location of farms was ineffective on CMT score(s) and bacteriology findings (Table 7). Çoban et al. (2007) reported that SCC did not show any discrepancies between different farms. They also concluded that the presence of similar SCC might be due to similar management strategies and location of farms.

In the present study, we found that the presence of no significant correlation between age and incidence of subclinical mastitis (Table 7). Şeker et al. (2000) stated the correlation between age and rate of CMT positive score. Sabuncuoğlu et al. (2003) and Rişvanlı and Kalkan (2002) reported the same findings that lack of correlation between age and rate of CMT positive score(s) as we found in our study. The present study also demonstrated that lactation number of cows was different but not statistically significant (Table 7). Sabuncuoğlu et al. (2003) reported the same observation as the present study stated, however, Çoban and Tüzemen (2007), Şeker et al. (2000) and Uzmay et al. (2003) found that lactation number was effective on the incidence of subclinical mastitis. The relationship between increasing lactation number and increasing SCC has been explained by the suppression of immune system associated with age, deformation capacity of milking machines on teat hence expedition of microorganisms towards teat canal (Çoban et al. 2007).

It was observed that lactation period was not effective on incidence of subclinical mastitis in the present study. The rate of isolated bacteria increased throughout nine months and then decreased (Table 7). Şeker et al. (2000) found that rate of CMT positive cows increased by progression of lactation period however it decreased between seven and nine months of lactation. Çoban et al. (2007) reported that high SCC was detectable at the beginning and end of lactation period. Çoban and Tüzemen (2007) observed that low incidence of subclinical mastitis at the beginning of lactation and the highest risk was at the end of lactation period. It is suggested that the discrepancies between the different studies may be associated with insufficient mastitis control programs, management conditions and seasonal factors.

When the breed factor on subclinical mastitis was evaluated (Table 7), it was seen that CMT and bacteriology scores were lower in Brown Swiss cows than those detected in other breeds but this difference was not significant. Sabuncuoğlu et al. (2003) and Çoban et al. (2007) reported that the breed was not an effective factor increasing subclinical mastitis, however Rişvanlı and Kalkan (2002) stated the higher SCC in Brown Swiss cows as compared to other breeds. It is suggested that breed is not causative for subclinical mastitis and all breeds have similar risk for the infection.

Milk samples (n:125) obtained from CMT positive mammary quarters were collected from right front (n:28), right rear (n:39), left front (n:25) and left rear (n:33) mammary quarters (Table 7), whereas the distribution of isolated bacteria was 32, 34, 26 and 36, respectively (Table 5). Accordingly, it was observed that CMT positivity and isolation of bacteria were higher in rear mammary quarters. This statistically non-significant finding was consistent with the results of Şeker et al. (2000). It is suggested that the high rate of CMT positivity in rear mammary quarters may be related to high milk yield of rear mammary quarters, anatomical structure and the closer distance of udders from the ground.

The bacteria isolated in the study were resistant against gentamycin, ceftiofur, cefquinome and enrofloxacin (Table 8). In addition, all bacteria except *Bacillus* spp. was sensitive against amoxycillin+clavulonic acid. Hadimli and Uçar (1999) reported the sensitivity of bacteria against amoxycillin+clavulonic acid, whereas Aydın et al. (1995) found that *S.aureus* showed resistance against ampicillin (57.40 %), kanamycin (28.57 %), enrofloxacin (10.71 %), neomycin (75.00 %), penicillin (82.40 %), streptomycine (46.42 %), tetracycline (67.85 %) and gentamycin (25.00 %). All these discrepancies possibly relate to regional differences of farms.

In conclusion, CMT of cows showed that positive results were obtained from one of every two cows. It is clear that farm localization, age, lactation number/period, breed and localization of mammary quarters of cows did not affect CMT scores and microbiological results. But functional problems of milking machine could directly affect the teat and udder health. Treatment of mastitis should be done following antibiotic susceptibility tests instead of ampiric therapy. It is suggested that mastitis keeps being a problem in those family-type farms, since the lack of information about mastitis control programs is exist. Therefore, it is needed that the breeders should be trained for management, feeding, milking hygiene and mastitis control programs.

Table 1: The percentage of cows housed in different farms in which at least one mammary quarter showed CMT positive reaction.

Tablo 1: Yerleşim yerlerine göre en az bir meme lobu CMT pozitif reaksiyon veren hayvan oranları

Location	**Breeds**						
	Holstein		**Brown-Swiss**		**Simmental**		**Total**
	n	**%**	**n**	**%**	**n**	**%**	
A	25	86,21	2	6,9	2	6,9	29
B	11	64,71	4	23,53	2	11,76	17
C	5	62,5	1	12,5	2	25	8
D	12	37,5	16	50	4	12,5	32
E	13	92,86	1	7,14	0	0	14
Total	**66**	**66,00**	**24**	**24,00**	**10**	**10,00**	**100**

Table 2: The number of examined cows and mammary quarters; the number and percentage of CMT positive and bacteria isolated cows and mammary quarters.

Tablo 2: Muayene edilen hayvan ve meme lobu sayısı, CMT pozitif ve izolasyon yapılan hayvan ve meme lobu sayıları ile oranları

Location	**Examined cows**	**CMT positive cows**		**Bacteria isolated cows (CMT + cows)**		**Examined udders**	**CMT positive udders**		**Bacteria isolated udders (CMT + mammary quarters)**	
		n	**%**	**n**	**%**		**n**	**%**	**n**	**%**
A	47	29	61,70	18	62,06	185	35	18,91	22	62,85
B	38	17	44,73	8	47,05	152	24	15,78	10	41,66
C	23	8	34,78	7	87,50	92	10	10,86	9	90,00
D	64	32	50,00	19	59,37	254	39	15,35	23	58,97
E	23	14	60,86	11	78,57	91	17	18,68	12	70,58
Total	**195**	**100**	**51,28**	**63**	**63,00**	**774**	**125**	**16,14**	**76**	**60,80**

Table 3: Percentage of CMT scores and bacterial isolation in CMT positive cows housed in different farms.

Tablo 3: CMT pozitif olan ineklerde yerleşim yerlerine göre CMT skor ve bakteriyel izolasyon oranları.

Location	**Percentage of CMT Scores**						**Percentage of isolated bacteria in CMT Scores**		
	+		++		+++		+	++	+++
	n	**%**	**n**	**%**	**n**	**%**	**%**	**%**	**%**
A	23	65,71	10	28,57	2	5,71	47,83	100	50
B	22	91,66	2	8,33	0	0	36,36	100	0
C	10	100	0	0	0	0	90	0	0
D	30	76,92	6	15,38	3	7,69	63,33	66,67	66,67
E	14	82,35	2	11,76	1	5,88	71,43	50	100
Total	**99**	**79,20**	**20**	**16**	**6**	**4,80**	**57,58**	**85**	**66,67**

Table 4: The number and percentage of microorganisms isolated from cows housed in different farms.

Tablo 4: **Y**erleşim yerlerine göre izole edilen mikroorganizmaların sayı ve oranları.

Location		**Microorganisms**														**Total isolation**
		S.uberis		***S.aureus***		***E.coli***		**CNS**		***Proteus* spp.**		***Bacillus* spp.**		***Candida* spp.**		
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*
A		10	28,57	1	2,86	9	25,71	1	2,86	0	0	1	2,86	13	37,14	35
B		1	5,88	3	17,65	6	35,29	2	11,76	0	0	1	5,88	4	23,53	17
C		2	14,29	0	0	5	35,71	5	35,71	0	0	0	0	2	14,29	14
D		8	20,51	1	2,56	9	23,08	9	23,08	3	7,69	0	0	9	23,08	39
E		4	17,39	0	0	8	34,78	8	34,78	0	0	0	0	3	13,04	23
Total	**n**	**25**		**5**		**37**		**25**		**3**		**2**		**31**		
	%	**19,53**		**3,9**		**28,9**		**19,53**		**2,34**		**1,56**		**24,21**		

Table 5: Distribution of microorganisms according to the mammary quarters' locations.

Tablo 5: Meme loblarına göre mikroorganizma sayı ve dağılımları.

Mammary quarters' location	**Microorganisms (n)**							**Total (n)**	**CMT positive mammary quarters (n)**	**Bacteria isolated from mammary quarters (n)**	**Bacteria isolated from mammary quarters/ CMT positive mammary quarters %**
	S.uberis	***S.aureus***	***E.coli***	***CNS***	***Proteus spp.***	***Bacillus spp.***	***Candida spp.***				
Right front	9	0	12	4	0	0	7	**32**	28	15	53.57
Right rear	5	2	8	6	2	1	10	**34**	39	24	61.53
Left front	3	2	7	6	0	1	7	**26**	24	17	70.83
Left rear	8	1	10	9	1	0	7	**36**	34	19	55.88

Note: There are some mix infections in some mammary quarters

Table 6: Mean, minimum and maximum values of lactation number, lactation period and age of cows having CMT positive mammary quarters related to the location of farms.

Tablo 6: Meme lobları CMT pozitif olan ineklerin yerleşim yerlerine laktasyon sayıları, laktasyon dönemleri ve yaşlarının minimum, maksimum ve ortalama değerleri.

Factors	**Location**	**Milk samples (n)**	**Mean±SE**	**Minimum**	**Maximum**
Lactation number	A	35	3,74±0,356	1	8
	B	24	3,38±0,499	1	8
	C	10	2,3±0,367	1	5
	D	39	3,51±0,369	1	8
	E	17	4,59±0,486	1	7
Lactation period	A	35	4,49±0,368	1	10
	B	24	5,25±0,817	1	12
	C	10	6,9±0,767	2	11
	D	39	6,49±0,618	1	12
	E	17	6,00±0,522	1	9
Age	A	35	6,37±0,428	3	10
	B	24	6,62±0,712	4	14
	C	10	4,6±0,499	3	8
	D	39	6,18±0,422	3	13
	E	17	7,24±0,673	3	12

Table 7: The effect of some factors on CMT and bacteriology results (The least squares mean ±SE).

Tablo 7: Bazı faktörlerin CMT ve mikrobiyolojik sonuçlara etkisi (en küçük kareler ortalaması±SE).

Factors		***Milk samples(n)***	***CMT scores***	***Bacteriologic isolation scores***
Location	A	35	1,434±0,165	0,58±0,129
	B	24	0,995±0,182	0,388±0,143
	C	10	1,106±0,234	0,797±0,183
	D	39	1,317±0,148	0,644±0,116
	E	17	1,332±0,215	0,811±0,168
Age (year)	3	17	1,706±0,498	0,196±0,389
	4	27	1,424±0,388	0,068±0,303
	5	15	1,317±0,326	0,342±0,254
	6	15	1,335±0,285	0,366±0,223
	7	8	1,494±0,293	0,273±0,229
	8	14	1,114±0,277	0,963±0,0216
	9	13	1,071±0,354	0,943±0,0276
	10	8	1,054±0,344	0,812±0,269
	12	3	0,908±0,48	0,128±0,375
	13	3	1,337±0,576	0,998±0,0001
	14	2	0,843±0,676	0,998±0,0002
Lactation Number	1	24	0,875±0,486	0.955±0,038
	2	27	0,985±0,402	0,959±0,031
	3	22	1,035±0,307	0,934±0,024
	4	11	1,266±0,288	0,464±0,225
	5	8	1,229±0,31	0,573±0,242
	6	17	1,403±0,3	0,383±0,235
	7	8	1,379±0,403	0,464±0,315
	8	8	1,721±0,426	0,42±0,333
Lactation Period (month)	1	13	1,232±0,201	0,674±0,157
	2	16	1,149±0,199	0,788±0,156
	3	9	1,125±0,247	0,848±0,193
	4	10	1,081±0,238	0,897±0,186
	5	10	0,97±0,228	0,603±0,178
	6	20	0,88±0,197	0,674±0,154
	7	17	1,405±0,178	0,649±0,139
	8	6	1,64±0,296	0,636±0,232
	9	7	0,875±0,262	0,921±0,205
	10	2	1,395±0,469	0,511±0,366
	11	5	1,42±0,335	0,295±0,262
	12	10	1,668±0,259	0,233±0,202
Breeds	Holstein	69	1,408±0,13	0,696±0,102
	Brown-Swiss	42	1,08±0,14	0,509±0,109
	Simental	14	1,222±0,203	0,727±0,159
Mammary quarters' locations	Right front	28	1,296±0,173	0,455±0,135
	Right rear	39	1,248±0,135	0,666±0,106
	Left front	25	1,31±0,166	0,882±0,129
	Left rear	33	1,093±0,139	0,573±0,108

CMT scores (+;1, ++;2, +++;3); Bacteriologic isolation scores (positive isolation;1, negative isolation;0); Breeds (Holstein;1, Brown-Swiss; 2, Simental; 3); Mammary Quarters' locations (right front; 1, right rear; 2 , left front; 3 , left rear; 4).

Table 8: Antibiotic sensitivity tests.

Tablo 8: Antibiyotik duyarlılık testleri.

Microorgansims	**Antibiotic Discs**						
	Amoxicillin + Clavulanic Acid	**Oxytetracycline**	**Penicillin**	**Gentamicin**	**Ceftiofur**	**Cefquinome**	**Enrofloxacin**
E.coli	100% (primary)	100% (secondary)	0%	0%	0%	0%	0%
S.uberis	100% (secondary)	100% (primary)	0%	0%	0%	0%	0%
S.aureus	100% (primary)	0%	0%	0%	0%	0%	0%
KNS	100% (tertiary)	100% (secondary)	100% (primary)	0%	0%	0%	0%
Proteus spp.	100% (primary)	100% (secondary)	0%	0%	0%	0%	0%
Bacillus spp.	0%	100% (primary)	0%	0%	0%	0%	0%

Note: The sensitivity is handled due to primary, secondary and tertiary zone diameters of antibiogram tests

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