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Research Article

An Evaluation of Subclinical Mastitis During Lactation in Anatolian Buffaloes

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Abstract: Subclinical mastitis in Anatolian buffaloes (n = 71) in Afyon, Turkey was evaluated using 1637 milk samples collected monthly for 9 months. Tests applied to milk samples included the California Mastitis Test (CMT), somatic cell count (SCC), and bacteriological examination.

The threshold limit for SCC was found as 130 × 10^3 cells/ml. Anatolian buffaloes are infected above this level and they are likely to be uninfected below this level. The highest rates of intramammary infection (IMI) per quarter (36.5%) and animal (69.1%) were encountered during the highest rainfall month of May. *Candida* spp., coagulase-negative staphylococcus (CNS) and *Staphylococcus aureus* and mixed infections were isolated at the rate of 41.91%, 20.59%, and 16.91% of quarters, respectively. Quarters infected with *S. aureus* (9.7%) were associated with CMT scores >0 and elevated SCC. No significant increase in CMT scores >0 and elevated SCC were observed during the period in which the incidence of *Candida* spp. was high. However, CMT (-) milk samples were determined to display higher SCC in autumn months when compared to other seasons. The microbial isolation rates in CMT (-) and CMT (+) quarters during the first 4 months of lactation were determined to be higher in comparison to other stages of lactation. The most sensitive antibiotic was amoxycillin+clavulonic acid.

As it has low numbers of infectious agents, bubaline milk is important for human welfare. However, higher infection rates were observed during rainy periods, during the first 4 months of lactation, after the 5th lactation, in late spring and early summer calvers, and in machine milked animals. In addition to SCC and CMT results, bacteriological examination could be carried out to identify mastitis.

Key Words: Aetiology, buffalo, California Mastitis Test, somatic cell count, subclinical mastitis

Anadolu Mandalarında Laktasyon Sürecinde Subklinik Mastitis Olgularının Değerlendirilmesi

Özet: Bu çalışmada, Afyon bölgesinde bulunan Anadolu mandalarında (n = 71) subklinik mastitis olgularının değerlendirilmesi amaçlandı ve bu mandalardan aylık periyotlar ile 9 ay süresince Kaliforniya Mastitis Test (CMT), somatik hücre sayısının (SHS) belirlenmesi ve bakteriyolojik değerlendirme amacıyla 1637 süt örneği alındı.

SHS için başlangıç değeri 130 × 10^3 hücre/ml olarak belirlendi. Meme lobu ve hayvan bazında en yüksek enfeksiyon oranı, bölgesel yağış miktarının en fazla olduğu Mayıs ayı içerisinde belirlendi. *Candida* türleri, koagulaz negatif stafilokoklar (CNS) ile *Staphylococcus aureus* ve miks enfeksiyonlar sırasıyla % 41,91, % 20,59 ve % 16,91 oranında izole edildi. *S. aureus* enfeksiyonları (% 9,71) en fazla oranda CMT >0 ve yüksek SHS'ye sahip olan süt örneklerinden izole edildi. *Candida* türlerinin yüksek oranda izole edildiği dönemlerdeki süt örneklerinin CMT >0 ve SHS sayılarında önemli bir artış gözlenmedi. Bununla birlikte CMT (-) süt örneklerindeki SHS'nin sonbahar aylarında diğer aylara göre önemli düzeyde yüksek olduğu saptandı. CMT (-) ve CMT (+) meme loblarındaki mikrobiyolojik üreme oranları laktasyonun ilk 4 aylık periyodu sürecinde sonraki dönemlere oranla oldukça yüksek olarak belirlendi. Manda sütlerinde izole edilen etkenlere karşı in vitro ortamda en etkili antibiyotiğin amoksasilin+klavulonik asit olduğu belirlendi.

Genel sonuç olarak, manda sütlerinde üreyen etken sayısının düşük olduğu ve insan sağlığı açısından önemli bir avantaj sağladığı belirlendi. Bununla birlikte yağışın en yüksek olduğu dönemlerde, laktasyonun ilk 4 aylık döneminde, 5. laktasyon periyodunu aşan mandalarda, ilkbahar sonu ile yaz başlangıcında doğum yapan mandalarda ve makina ile sağım yapılan mandalarda süt kalitesinde önemli oranda azalma olduğu tespit edildi. Mastitisin belirlenmesi için SHS ve CMT sonuçlarının yanında bakteriyolojik değerlendirmenin de yapılması gerektiği belirlendi.

Anahtar Sözcükler: Etiyoloji, manda, Kaliforniya Mastitis Test, somatik hücre sayısı, subklinik mastitis

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Introduction

Buffaloes, recognized to have economic significance among livestock animals in terms of milk and meat yields as well as work purposes, are bred in the tropical and subtropical regions of Asia, South America, North Africa, all Mediterranean countries excluding France, certain Central European countries, and Australia (1). Anatolian buffaloes are classed as a river type belonging to a Mediterranean group (2).

Although mastitis is seldom encountered among dairy buffaloes in comparison to dairy cattle on small farms, infections of the udder may present a serious problem in large intensive herds in India and Pakistan (3-5). The rate of subclinical mastitis is reported to be 5.8% in buffaloes in Brazil and 6.7% in quarters in Pakistan according to California mastitis test (CMT) results (3,6). Wanasinghe (4) reported the rates of mastitis cases diagnosed by means of CMT in a comparative study carried out on both buffaloes and cattle as 43% and 66% of animals and 20% and 44% of quarters, respectively, and concluded that buffaloes are more resistant to udder infections than cattle. Alaçam et al. (7) reported the distribution of subclinical mastitis as 4.7%-16.3% in buffaloes and 6.7%-18.8% in cattle, according to CMT results.

To our knowledge, no previous studies have reported the use of somatic cell count (SCC), CMT, and the isolation of infectious agents in Anatolian buffaloes held in family holdings on a regional basis. This study determined the prevalence of subclinical mastitis, the causative agents of such cases, and the sensitivity of these pathogens to antibiotics, with regard to stages of lactation, lactation number, season and milking techniques in Anatolian buffaloes bred in Afyon province, Turkey.

Materials and Methods

Herds and Animals

The present study was carried out on 71 buffaloes (281 mammary quarters), aged 3-12 years, in different stages of lactation, and held in either a public farm (Kocatepe Agricultural Research Institute) or private farms located in Afyon province. The public farm had 40 animals with 943.2 ± 276.2 kg average milk yield per lactation (milk yield varied between 350-1580 kg/lactation). There were 7 animals on private farms 1 and 3, 5 animals on private farm 2, and 6 animals on private farms 4 and 5. Their average milk yield per lactation was 741.3 ± 191.7 kg (milk yield varied between 180-935 kg/lactation). Stage of lactation and gestation period of buffaloes included in this study varied between 82-390 days and 305-321 days, respectively. All the buffaloes were housed in stables during harsh winter conditions, and allowed to move freely in open paddocks in spring and summer. Animals on the public farm were milked by machine while those on private farms were hand-milked. Machine milking and hand-milking were performed twice a day (0900 and 1500 h). Animals included in the presented study were determined to be negative for tuberculosis and *Brucella* infections.

The Collection of Milk Samples, CMT, SCC, Bacteriological Examination and Antibiotic Sensitivity Tests

Milk samples were collected from buffaloes in the mornings. The CMT, SCC, and bacterial examination were carried out for 9 months, taking into consideration their last dates of calving. The CMT test was carried out in each mammary quarter of all buffaloes. CMT results were evaluated as negative (-), trace (T), (+1), (+2), and (+3) (8). SCC was measured for each quarter by means of the direct microscope method (9). Quarter foremilk samples for bacteriological examination were collected under aseptic conditions. All milk samples were transported to the laboratory in a cool chain. Milk samples were centrifuged at 3000 rpm for 10 min. A 0.1 ml aliquot was taken from each milk sample and inoculated onto blood agar containing 7% sheep blood, MacConkey agar, and Sabouraud dextrose agar medium. Blood agar and MacConkey agar plates were incubated under aerobic conditions at 37 ºC for 24-48 h. Sabouraud Dextrose agar plates were incubated under aerobic conditions at 24 ºC for 5-7 days. After incubation each different bacterial colony was examined macroscopically (colony morphology, haemolysis) and microscopically (Gram's stain). Each different colony was subcultured onto blood agar media, containing 7% sheep blood for further characterisations. Identification of all isolates was performed using standard biochemical tests according to standard manuals (10-12). All fungal colonies were examined macroscopically and microscopically as described by Quinn et al. (11).

An antibiotic susceptibility test was performed using the disk diffusion method on Mueller-Hinton Agar according to the National Committee of Clinical Laboratory Standards (NCCLS) (13). Pure colonies from

the blood agar medium, incubated at 37 °C for 18 h, suspended in 2 ml sterile saline to a density approximately equal to McFarland Opacity Standard No 0.5. A dry cotton wool swab was placed into the suspension and excess liquid was expressed against the inside of the tube. The bacterial suspension was then inoculated onto Mueller-Hinton agar with the swab in such a way that the whole surface of the agar was covered. The plates were incubated at 37 °C for 24 h. The results were recorded by measuring the inhibition zone diameter according to the interpretive standards of NCCLS (13).

Statistical Analyses

The chi-square test was used for the establishment of the relationships between the stage of lactation, season, lactation number, and milking technique with microbial growth. The variance analyses of SCC among the groups were carried out according to 1-way analysis of variance method (Duncan test). Receiver operating characteristic (ROC) curve analysis was also used to determine the optimal cut-off points having the highest sensitivity and specificity for determination of subclinical mastitis.

Results

The relationships between the number and percentage of infected mammary quarters and the CMT scores and SCC values

Candida species were isolated from 41.91% of the quarters and were the predominant microorganism isolated from the milk samples. Coagulase-negative staphylococci were the second most common organism isolated and were found in 20.59% of the samples, with *S. aureus* and mixed infections found in 16.91% of the samples (Table 1). Amongst the infectious agents that were isolated from CMT (T) and CMT (+) samples, *S. aureus* was determined to have the highest incidence isolated from 9.7% of the quarters (Table 1).

In buffaloes, distribution of the number of infected mammary quarters varied according to seasons (Table 2). The highest prevalence of infection per animal and per mammary quarter was determined in May as 69.1% and 36.5%, respectively. With regard to all seasons, percentages of infected mammary quarters per animal and per mammary quarter were determined as 16.8% and 8.3%, respectively. Results from CMT measurements of mammary quarters show a significant difference ($P < 0.05$) between microbial growth and season (Table 2). The growth of infectious agents in CMT (-) and CMT (+) animals were determined to increase in spring in comparison to other seasons. Analysis of seasonal CMT and SCC data indicates that the SCC of CMT (-) mammary quarters showed a significant increase in autumn months when compared to other months (Table 3).

All *S. aureus* infections and 64.9% of the isolated *Candida* species were encountered in the holding in which machine milking was performed. Also, the number of mammary quarters defined as CMT (T), and CMT (+1, +2, +3) was greater for the machine-milked buffaloes compared to the hand-milked buffaloes (Table 4).

Table 1. Infectious agents isolated from mammary quarters and their calculated percentages.

	All agents	CMT >0	Mean SCC (All agents)
Causative agent	Number (%)	Number (%)	(cells/ml $\times 10^3$)
S. aureus	23 (16.91)	20 (9.71)	2,957 ± 3,332
CNS	28 (20.59)	11 (5.34)	231 ± 320
Bacillus spp	2 (1.47)	1 (0.49)	112 ± 88
Penicillum spp	3 (2.21)	1 (0.49)	131 ± 189
Candida spp	57 (41.91)	13 (6.31)	279 ± 1,331
S. aureus+*Bacillus* spp	1 (0.74)	1 (0.49)	1,255
S. epidermidis+*Bacillus* spp	1 (0.74)	0	15
S. aureus+*Candida* spp	5 (3.68)	5 (2.43)	1,361 ± 952
S. epidermidis+*Candida* spp	14 (10.29)	9 (4.37)	840 ± 1,398
Bacillus spp+*Candida* spp	2 (1.47)	1 (0.49)	97 ± 74
With microbial growth	136 (100)	62 (30.10)	820 ± 1,952
Without microbial growth	1501	144 (69.90)	107 ± 312

Table 2. The seasonal distribution of infectious agents according to CMT findings observed in mammary quarters of buffaloes.

	Total (n)	CMT (-) (%)	Growth (%)	CMT (T) (%)	Growth (%)	CMT (+1,+2,+3) (%)	Growth (%)	Mean Rainfall (mm)	Mean Wind Speed (m/second)
Spring	221	186 (84.16)	48[a] (25.81)	17 (7.69)	6[a] (35.29)	18 (8.14)	11[a] (61.11)	54	2.1
Summer	669	602 (89.99)	13[bc] (2.16)	28 (4.19)	8[a] (28.57)	39 (5.83)	14[ab] (35.9)	20.4	2.2
Autumn	629	552 (87.76)	6[c] (1.09)	34 (5.41)	5[a] (14.71)	43 (6.84)	13[b] (30.23)	33.8	1.6
Winter	118	91 (77.12)	7[d] (7.69)	13 (11.02)	3[a] (23.08)	14 (11.86)	2[b] (14.29)	48.8	6.6
P			**		•		*		

•: Insignificant difference between groups : *P < 0.05 : **P < 0.001
[a,b,c,d]; Significant difference between values symbolised with different letters within the same column

Table 3. The seasonal distribution of SCC (cells/ml × 10^3) according to CMT findings observed in mammary quarters of buffaloes.

	Total (n)	(-)	SCC	(T)	SCC	(+1)	SCC	(+2)	SCC	(+3)	SCC
Spring	221	186 (84.16)	45[a] ± 36	17 (7.69)	299[a] ± 238	11 (4.98)	500[a] ± 443	6 (2.71)	1,178[a] ± 264	1 (0.45)	10,000
Summer	669	602 (89.99)	51[ac] ± 46	28 (4.19)	289[a] ± 326	23 (3.44)	489[a] ± 538	13 (1.94)	1,570[a] ± 691	3 (0.45)	1,272 ± 1,601
Autumn	629	552 (87.76)	69[b] ± 48	34 (5.41)	273[a] ± 207	22 (3.5)	493[a] ± 237	16 (2.54)	1,977[a] ± 1,319	5 (0.79)	1,878 ± 2,497
Winter	118	91 (77.12)	50[c] ± 37	13 (11.02)	384[a] ± 365	12 (10.17)	489[a] ± 276	2 (1.69)	752[a] ± 152	-	-
P			**		•		•		•		

•: Insignificant difference between groups **: P < 0.001
[a,b,c]; Significant difference between values symbolised with different letters within the same column

The sensitivity, specificity of SCC and CMT results

The rates of maximum sensitivity, specificity, cut-off point of SCC, and area under the ROC curve are shown in Table 5 and Figure (a) and (b). The rates of sensitivity and specificity of CMT scores are given in Table 6.

The Distribution of CMT scores, SCC values and Microbial Growth According to the Stage and Number of Lactation

An analysis of CMT findings observed in the mammary quarters of the buffaloes indicates a significant correlation

Table 4. The number of infected mammary quarters, SCC (cells/ml ×10^3), and CMT values in the case of machine and hand milking.

	Total number of buffaloes	Number of mammary quarters	Infected mammary quarters				Total number of infected mammary quarters	SCC	CMT T,+1,+2,+3
			S. aureus	CNS	*Candida* species	Other			
Family holdings (Hand milking)	31	788	0	17	20	7	44	79 ± 228	43 (5.46%)
Buffalo breeding public farm (Machine milking)	40	849	23	11	37	21	92	242 ± 854	163 (19.20%)

Table 5. The rates of maximum sensitivity and specificity (%), cut-off point of SCC (cells/ml × 10^3), and area under the ROC curve.

	Sensitivity	Specificity	Cut-off point	Area under the ROC curve
Any infection compared with no growth (Figure (a))	51.5	87.4	130	0.65
Infections with *S.aureus* compared with no growth or the other pathogens (Figure (b))	89.7	94.9	375	0.97

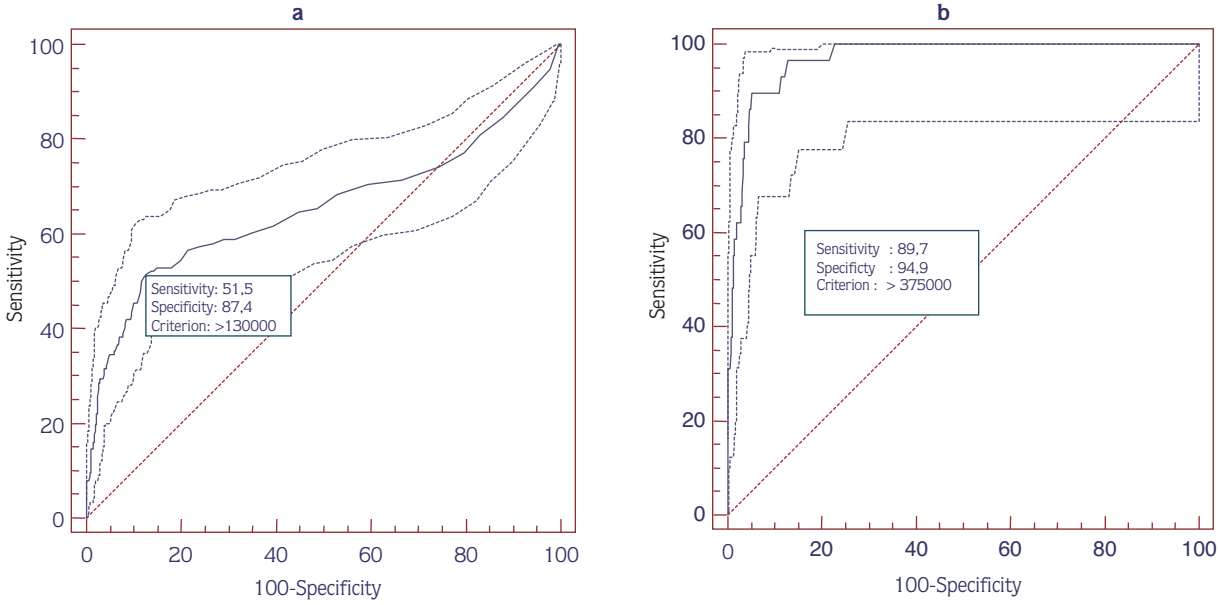

Figure. ROC curve for any infection compared with no growth (a) and for infections with *S. aureus* compared with no growth or the other pathogens (b).

Table 6. The rates of sensitivity and specificity of CMT >0.

		CMT > 0
Any infection compared with no growth	Sensitivity Specificity	45.6 90.4
Infection with major pathogen compared with no growth or minor pathogen	Sensitivity Specificity	89.7 88.8

($P < 0.05$) between the infectious agents and stage of lactation. Although not statistically significant, higher numbers of infectious agents according to CMT findings were observed within the first 4 months of lactation. A significant decrease in microbial growth in mammary quarters defined as CMT (-) and CMT (+) was seen following the 5th month of lactation (Table 7). A significant difference ($P < 0.001$) was determined

Table 7. The distribution of infectious agents over stages of lactation according to CMT findings observed in the mammary quarters of buffaloes.

	Total (n)	CMT(-) (%)	Growth (%)	CMT(T) (%)	Growth (%)	CMT (+1,+2,+3) (%)	Growth (%)
1st-2nd months of lactation	341	300 (87.98)	27[a] (9.00)	18 (5.28)	8[a] (44.44)	23 (6.74)	14[a] (60.87)
3rd-4th months of lactation	432	380 (87.96)	28[a] (7.37)	16 (3.70)	5[a] (31.25)	36 (8.33)	14[a] (38.89)
5th-6th months of lactation	410	369 (90.00)	15[b] (4.07)	24 (5.85)	4[a] (16.67)	17 (4.15)	6[ab] (35.29)
≥7th month of lactation	454	382 (87.14)	4[c] (1.05)	34 (7.49)	5[a] (14.71)	38 (8.37)	6[b] (15.79)
P			**		•		*

•: Insignificant difference between groups *: P < 0.05 **: P < 0.001
a,b,c; Significant difference between values symbolised with different letters within the same column

between infectious agents and lactation number according to the CMT findings observed in the mammary quarters of buffaloes. Our data indicate that CMT (T) and CMT (+) showed significant increase, particularly after the 5th lactation (Table 8). Although no significant difference was observed in the infectious agent over the stages of lactation, all *S. aureus* (100%) isolated throughout the study period and most of the *Candida* species (33.3%) were isolated from animals in their 7th and 1st lactations respectively. Analysis of CMT findings and SCC with regard to the stage of lactation, shows a significant increase (P < 0.001) in the SCC values of CMT (-) mammary quarters during late lactation (Table 9). Lower SCC values of mammary quarters displaying CMT (-) findings were observed during the first 2 lactations. A significant difference was observed in the SCC values of

Table 8. The distribution of infectious agents with regard to the number of lactations according to the CMT findings observed in the mammary quarters of buffaloes.

	Total (n)	CMT (-) (%)	Growth (%)	CMT (T) (%)	Growth (%)	CMT (+1,+2,+3) (%)	Growth (%)
1st-2nd lactation	720	659 (91.53)	33[ab] (5.01)	21 (2.92)	3[ab] (14.29)	40 (5.56)	5[a] (12.50)
3rd-4th lactation	472	397 (84.11)	13[a] (2.75)	45 (9.53)	6[a] (13.33)	30 (6.36)	5[a] (16.67)
≥5th lactation	445	375 (84.27)	28[b] (7.47)	26 (5.84)	13[b] (50.00)	44 (9.89)	30[b] (68.18)
P			*		*		**

*: P < 0.05 **: P < 0.001
[a,b]; Significant difference between values symbolised with different letters within the same column

Table 9. The distribution of SCC (cells/ml × 10^3) with regard to the stage of lactation according to the CMT findings observed in the mammary quarters of buffaloes.

	Total (n)	(-)	SCC	(T)	SCC	(+1)	SCC	(+2)	SCC	(+3)	SCC
1^{st}-2^{nd} months of lactation	341	300 (87.98)	$37^{a} \pm 27$	18 (5.28)	$346^{a} \pm 314$	12 (3.52)	$550^{a} \pm 478$	8 (2.35)	$1,936^{a} \pm 614$	3 (0.88)	6,81 ± 2,775
3^{rd}-4^{th} months of lactation	432	380 (87.96)	$47^{b} \pm 35$	16 (3.70)	$251^{a} \pm 140$	15 (3.47)	$584^{a} \pm 637$	16 (3.70)	$1,591^{a} \pm 929$	5 (1.16)	8,200 ± 1,095
5^{th}-6^{th} months of lactation	410	369 (90.00)	$61^{c} \pm 45$	24 (5.85)	$247^{a} \pm 114$	11 (2.68)	$415^{a} \pm 178$	5 (1.22)	$2,310^{a} \pm 1,838$	1 (0.24)	6,250
$\geq 7^{th}$ month of lactation	454	382 (87.14)	$78^{d} \pm 58$	34 (7.49)	$298^{a} \pm 299$	30 (6.61)	$459^{a} \pm 251$	8 (1.76)	$948^{a} \pm 164$	-	-
P			**		•		•		•		

•: Insignificant difference between groups **: P < 0.001

^{a,b,c,d}: Significant difference between values symbolised with different letters within the same column

mammary quarters displaying findings typical of CMT (+2) during the first 2 lactations in comparison to the 3^{rd} and 4^{th} lactations (Table 10).

The most effective antibiotic in vitro against *S. aureus*, *S. epidermidis* and *Bacillus* species was determined to be amoxycillin + clavulonic acid which showed a 92% sensitivity against these bacteria. The order of sensitivity was determined to be as follows, based on the indicated percentages: oxytetracycline 42%, danofloxacin 37%, fluorfenicole 36%, streptomycin 17%, enrofloxacin 13%, ampicillin and gentamycin 4%. The isolated microorganisms were determined not to be sensitive to penicillin G and erythromycin.

Discussion

The present study revealed that despite the high mean rainfall and wind speed values in winter, microbial growth in CMT (+) milk samples reached significantly high rates (36.5% and 69.1% per mammary quarter and per animal, respectively) during spring, particularly in May, the highest rainfall month. According to Turkish State Meteorological Service, the mean rainfall and wind speed rates of the region throughout the study period were highest in spring and winter, respectively (14). Our findings differ from the study of Alaçam et al.(7), which indicated that the rate of subclinical mastitis in buffaloes of the same region was highest in summer months.

Table 10. The distribution of SCC (cells/ml × 10^3) with regard to the lactation number according to the CMT findings observed in the mammary quarters of buffaloes.

	Total (n)	(-)	SCC	(T)	SCC	(+1)	SCC	(+2)	SCC	(+3)	SCC
1^{st}-2^{nd} lactation	720	659 (91.53)	$46^{a} \pm 33$	21 (2.92)	$226^{a} \pm 173$	26 (3.61)	$463^{a} \pm 507$	14 (1.94)	$1,983^{a} \pm 837$	-	-
3^{rd}-4^{th} lactation	472	397 (84.11)	$64^{b} \pm 47$	45 (9.53)	$309^{a} \pm 277$	21 (4.45)	$483^{a} \pm 286$	7 (1.48)	$1,017^{b} \pm 559$	2 (0.42)	6,500 ± 1,414
$\geq 5^{th}$ lactation	445	375 (84.27)	$68^{b} \pm 57$	26 (5.84)	$312^{a} \pm 323$	21 (4.72)	$511^{a} \pm 315$	16 (3.60)	$1,550^{ab} \pm 1,153$	7 (1.57)	7,812 ± 1,869
P			**		•		•		*		

•: Insignificant difference between groups *: P < 0.05 **: P < 0.001

^{a,b}: Significant difference between values symbolised with different letters within the same column

However Paranjape and Das (15) indicated that the prevalence of mastitis in buffaloes in Bombay was highest during the monsoon season, which is in line with our findings. İzgür (16) has reported climatic conditions including humidity above 80%, wind speed exceeding 0.2 m/s during summer, and 0.15 m/s in winter at the level of the udder, and wide differences between night and day temperatures to be influential upon the development of mastitis. Other studies (17,18) have presented a calving period concentrated between April and August in Anatolian buffaloes. Furthermore, we observed that an increased risk of mastitis for Anatolian buffaloes also mainly concentrated between April and August during the first month of lactation.

CMT trace and positive reactions were observed in 12.6% of all the mammary quarters in this study. Subclinical mastitis due to CMT positive reactions in mammary quarters of buffaloes was reported to be 6.7% in Pakistan (3). Alaçam et al. (7) have reported the rate of subclinical mastitis cases diagnosed in buffaloes according to CMT findings to vary between 4.7% and 16.3% in Turkey. The same researchers have isolated micro-organisms from 62.5% of the milk samples collected from buffaloes with subclinical mastitis. This percentage corresponds with the reports of the aforementioned researchers. Bacterial examination carried out on milk samples collected from mammary quarters defined as CMT trace and CMT positive from buffaloes included in this study has revealed microbial growth in 30.1% of samples. This percentage has been found to be lower than that previously reported by Alaçam et al. (7). The reason for this result has been suggested to be that these Anatolian buffalo were kept on a single public farm. Schalm et al. (8) have reported that although subclinical mastitis can be diagnosed by means of CMT, it may not always be possible to isolate all of the microorganisms causing infection. Furthermore, factors including age, trauma, lesions of the teats, metabolic diseases, stress, genetic factors, management and feeding of animals, and viral mastitis are considered to influence yield of trace or positive CMT results (19).

Among the infectious agents isolated from CMT (T) and CMT (+) mammary quarters, *S. aureus* had the highest incidence with 9.7% of quarters infected (Table 1). No marked increase was observed in CMT >0 and SCC during the period in which the infection rate of *Candida* species was determined to be high. However SCC was highest in CMT (-) milk samples during the autumn months compared to other seasons. Furthermore, both CMT and SCC values were observed to be high in public farms performing machine milking. Similar to the case in dairy cattle, a positive correlation was determined between CMT (+) scores and *S. aureus* infections and SCC in buffaloes.

It was considered that SCC below 100×10^3 cells/ml was normal, and SCC above 200×10^3 cells/ml was abnormal and indication of mastitis in cows (20,21). Dhakal (20) reported the SCC of milk from clinically normal Murrah buffaloes was 151×10^3 cells/ml in his mastitis study carried out in Nepalian and Indian conditions in 400 mammary quarters from 60 buffaloes. In the present study, the cut off point of SCC of milk from Anatolian buffaloes infected with any pathogen and infected with *S. aureus* was found to be 130×10^3 cells/ml and 375×10^3 cells/ml respectively (Table 5). This SCC threshold limit may be a useful detection criterion for subclinical mastitis in Anatolian buffaloes.

The specificity for mammary quarters infected with any pathogen was 87.4% for SCC and 90.4% for CMT >0. In addition, the specificity for mammary quarters infected with *S. aureus* was 94.9% for SCC and 88.8% for CMT >0. These high values showed that both SCC and CMT scores could be used for identifying healthy mammary quarters in Anatolian buffaloes.

While the sensitivity for mammary quarters infected with any pathogen was found 51.5% for SCC and 45.6% for CMT >0, the sensitivity for *S. aureus* infected mammary quarters was found 89.7% for both SCC and CMT. According to our findings, although SCC and CMT >0 data for *S. aureus* were considered indicative of mastitis due to the high sensitivity, the results for the mammary quarters infected with any pathogen could not be used alone because of the medium or low sensitivity values. Therefore, bacteriological examinations should be carried out together with SCC and CMT scores for detection in Anatolian buffaloes.

In a study carried out in the Sangali region of India which examined the effects of machine milking on udder health, Thomas (22) reported higher levels of mastitis in large herds. This study determined that SCC values and CMT (T,+1,+2,+3) scores of mammary quarters in machine milked buffaloes to be higher compared to hand milked animals. This may be due to milking personnel

lacking information on the proper and hygienic use of milking machines. It is also possible that because of the smaller cisternal fraction of buffaloes in comparison to dairy cattle, attachment of the milking cluster to the teats without pre-stimulation and milk let-down may result in milking empty udders and in turn cause higher teat penetration into the teat cup. For this reason, in order to prevent stress and irritation of the teats caused by the lack of milk flow at high vacuum, it has been reported that milk ejection should be stimulated prior to milking in all buffaloes (22).

The rate of microbial growth in CMT (-) and CMT (+) mammary quarters during the first 4 months of lactation was determined to be higher in comparison to the other stages of lactation. However, the SCC values of CMT (-) mammary quarters were observed to increase over advanced stages of lactation. Despite reports (23,24) of significant influence of stage of lactation on cases of subclinical mastitis and new infections in dairy cattle, the number of studies carried out on buffaloes is limited (6, 25). Singh and Ludri (25) have reported stage of lactation not to have any effect on the development of mastitis in buffaloes. Possible reasons for the high level of microbial isolation during the early lactation period may include the high level of stress in buffaloes following calving, buffaloes not being separated during the calving and colostrum periods, lack of hygienic treatment prior to and after milking and particularly the contamination of the udders due to the swampy and muddy area in which the buffaloes were kept (26).

This study has revealed a high percentage (33.3%) of *Candida* species isolated from animals in their first lactation, whereas all of the *S. aureus* infections were determined in animals in their 7^{th} lactation. The animals from which *Candida* species were isolated had not received any antibiotics and were not subjected to any management system that differed from those applied to the other animals in this study. A marked decline was observed in the growth rate of *Candida* species following the 4^{th} month of lactation. This may indicate that the rainy season (around May) in Turkey could be associated with the initiation of *Candida* species infection in the first month of lactation.

The rate of microbial growth in CMT positive mammary quarters following the 5^{th} lactation was determined to be higher compared to other lactation stages in buffaloes. No other studies have provided data on the relationship between the number of lactations and development of mastitis in buffaloes. The rate of mastitis cases is known to be closely related to the number of lactations in cattle. The decline in the strength of natural resistance of the mammary tissue due to aging in dairy cows contributes to predisposition to the development of mastitis, in association with many other factors. It has been reported that the number of animals diagnosed with mastitis increases as the number of old animals within a herd increases (16,27). Data from these studies has suggested that an increase in the prevalence of infection with advanced lactation numbers and age as well as the damage to the glandular tissue of the udder is caused by previous infections (27).

The most effective antibiotic against infectious agents isolated from milk samples pertaining to buffaloes under in vitro conditions was determined to be amoxicillin + clavulanic acid with a sensitivity rate of 92%. This rate has been found to be similar to that reported by Alaçam et al. (7) who have also carried out a study on a holding located in the same region.

It was concluded that Anatolian buffaloes bred in Afyon region had lower levels of subclinical mastitis than compared to previous studies, and their milk was of high quality with the low level of SCC (130×10^3 cells/ml). Above this level Anatolian buffaloes are infected or below this level Anatolian buffaloes are likely to be uninfected. However, the growth rate of pathogen microorganisms and SCC values was determined to increase in holdings in which machine milking was performed, during months with highest rainfall, in late spring and early summer calvers and after the 5^{th} lactation period. Somatic cell counts were observed to increase without microbial growth as the stage of lactation progressed. Therefore, bacteriological examinations should be carried out together with SCC and CMT scores for the detection of mastitis in Anatolian buffaloes.

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