HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

The Relationship Among the Hygiene Score, Bedding Type and the California Mastitis Test Score in Family-Type Dairy Farming

Adil Uztemur[1,a,*], Abdülkadir Orman[2,b]

[1]Republic of Türkiye, Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks, Şanlıurfa, Türkiye.

[2]University of Bursa Uludag, Faculty of Veterinary Medicine, Department of Animal Science, Bursa, Türkiye.

[a]ORCID: 0000-0003-4866-2663

[b]ORCID: 0000-0001-9138-4422

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***Correspondence:** Adil Uztemur

Republic of Türkiye, Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks, Şanlıurfa, Türkiye

e-mail: vet.hek.adiluztemur@gmail.com

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Abstract: It is known that subclinical mastitis has a significant effect on milk yield losses. The hygiene of both the barn and the cow plays an important role in the etiology of subclinical mastitis. In cases where the hygiene score (HS) worsens, the incidence of subclinical mastitis increases. The presented study aimed to determine the relationship between the diagnosis of subclinical mastitis with the California Mastitis Test (CMT) and the Hygiene score (HS). The study was conducted on 80 dairy cows of different breeds from small family-type dairy farms (n=10). Hygiene scores (HS) were recorded in different parts of the body of each animal and the incidence of subclinical mastitis was investigated with CMT in each mammary lobe. When the bedding materials in the barn were compared with the hygiene score (HS); a significant relationship was found between the bedding material of the legs (P=0,037) and belly (P=0,025), respectively. However, no significant relationship was found between the CMT test and the legs and belly (P>0,05). It has been determined that the positivity rate in CMT test results increases in animals with increased hygiene scores (HS). As a result, the relationship between the CMT test and subclinical mastitis and hygiene scores (HS) is thought to be insufficient. No significant relationship was found between the hygiene score (HS) of the rear and animals with positive CMT test results in any mammary lobe (P>0.05). It has been determined that the positivity rate in CMT test results increases in animals with increased hygiene scores (HS). As a result, the relationship between CMT test and subclinical mastitis and hygiene scores (HS) is thought to be insufficient. It would be more effective if the relationship between subclinical mastitis and hygiene score (HS) is supported by Somatic Cell Count (SCC) determination in addition to the CMT test.

Keywords: *CMT test, Dairy farm, Hygiene score, Subclinical mastitis.*

Aile Tipi İşletmelerde Hijyen Skoru, Yataklık Tipi ile California Mastitis Test Skoru Arasındaki İlişki

Özet: Subklinik mastitisin süt verim kayıplarında önemli düzeyde etkili olduğu bilinmektedir. Subklinik mastitisin etiyolojisinde hem ahırın hemde ineğin hijyeni önemli bir rol oynar. Hijyen skorunun kötüleştiği durumlarda subklinik mastitis insidası artar. Sunulan bu çalışmada Kaliforniya Mastitis Testi (CMT) ile subklinik mastitisin belirlenmesiyle hijyen skoru (HS) arasında ilişkinin belirlenmesi amaçlandı. Küçük aile tipi işletmelerde (n=10) bulunan farklı ırklara ait 80 adet sağmal ineklerde araştırma gerçekleştirildi. Her bir hayvanda vücudun farklı bölgelerinde hijyen skoru (HS) kaydedilerek her bir meme lobunda CMT ile subklinik mastitis insidansı araştırıldı. Barınak içerisindeki altlık materyalleri hijyen skoru (HS) ile karşılaştırıldığında; sırasıyla ayakların (P=0,037) ve karın bölgesinin (P=0,025) altlık materyali ile aralarında anlamlı bir ilişki olduğu bulunmuştur. Ancak CMT testi ile bacaklar ve karın bölgesi ile aralarında anlamlı bir ilişki bulunmamıştır (P>0,05). Herhangi bir meme lobunda pozitif CMT test sonucu çıkan hayvanlar ile sağrının hijyen skoru (HS) arasından anlamlı bir ilişki bulunmamıştır (P>0,05). Hijyen skoru (HS) artan hayvanlarda CMT testi sonuçlarında pozitiflik oranı arttığı tespit edilmiştir. Sonuç olarak CMT testi ile subklinik mastitis ile hijyen skoru (HS) arasındaki ilişkinin yetersiz olduğu düşünülmektedir. Subklinik mastitis ile hijyen skoru (HS) arasındaki ilişki için CMT testine ek olarak Somatik Hücre Sayısı (SHS) tespiti ile desteklenirse daha etkili olacaktır.

Anahtar Kelimeler: *CMT testi, Hijyen skoru, Subklinik mastitis, Süt işletmeciliği.*

Introduction

It is known that the most significant economic losses due to milk yield are losses due to mastitis in lactating dairy cows (Abdeen et al., 2021; de Campos et al.,2023; Ijaz et al., 2021). It has been reported that mastitis has many causes (Cobirka et al., 2020). Mastitis varies depending on several factors, including animal breed, age, lactation number, lactation duration, and environmental conditions. For example, Holstein cows are more prone to mastitis than Brown Swiss (Çoban and Tüzemen, 2007). The incidence of mastitis in older animals is higher than in younger animals (Rişvanlı and Kalkan, 2002). Mastitis is divided into two groups: clinical and subclinical (Cobirka et al., 2020; Han et al., 2022). While clinical mastitis is easier to detect by external symptoms, subclinical mastitis continues for a long time without clinical symptoms and goes unnoticed causing yield losses (Ijaz et al., 2021). Clinical mastitis findings are detected by redness in the mammary gland, edema, pain, hardness, and the presence of a vial in milk. Detection methods, including Somatic Cell Count (SCC), measurement of milk electrical conductivity, microbiological examinations, biochemical methods, PCR, and enzyme assays, are used in the diagnosis of subclinical mastitis (Abdeen et al., 2021; Anwar et al., 2022; Cantekin et al., 2015; Özenç, 2019). SCC determination methods are the most commonly used method in practice. Increasing the number of somatic cells (over 400,000 cells/mL) increases the incidence of mastitis in milk. It has been reported that the majority of milk yield loss is due to subclinical mastitis (Çelik and Akçay, 2024; De Graves and Fetrow 1993; Yalçın et al., 2000). Furthermore, SCC is a significant criterion for determining milk quality (Feng et al., 2021; Şafak et al., 2022). CMT is known to be the most common test for identifying SCC in milk and diagnosing subclinical mastitis (dos Santos et al., 2020). Also, they reported that there is a loss of milk yield for each unit increase in the positivity level in CMT (Ayvazoğlu Demir and Eşki, 2019). In addition, the hygiene score of the udder, depending on environmental effects, provides information about mastitis. Unhygienic barn conditions and non-compliance with milking rules increase the incidence of mastitis. The number of somatic cells in milk also increases in animals with increased hygiene scores (Akdag et al., 2017; Çelebi and Akdağ, 2022; Sharif and Muhammad, 2008). It has been reported that the rate of mastitis increases due to high hygiene scores (Mıtev et al., 2013). There are many factors affecting the hygiene score of cows. These include: type of barn, barn conditions, type of bedding, bedding material, grouping, etc. Many factors can be listed. Bedding type and bedding materials are among the important factors. For example, it has been reported that the body parts of cows in bedding types with dry and soft permeable bedding materials are cleaner, while the body parts of cows housed in impermeable, hard, wet bedding are dirtier (Fulwider et al., 2007; Hultgren and Bergsten, 2001; Koçyiğit and Tüzemen, 2015).

Based on this information, we decided to investigate whether there is a relationship between subclinical mastitis using only the CMT test, depending on the hygiene score, in dairy cows in family-type dairy farming.

Material and Methods

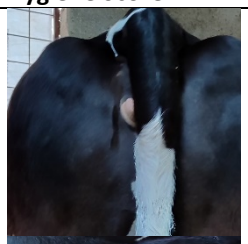
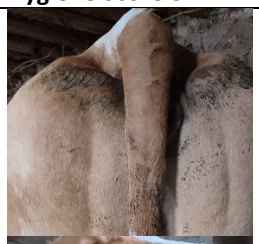
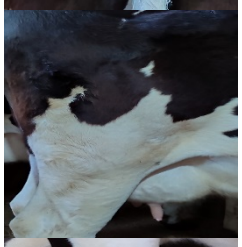
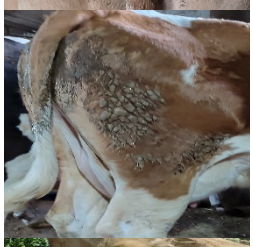
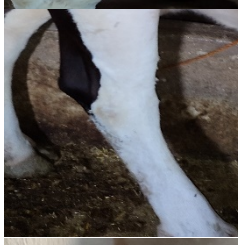
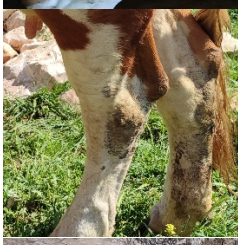
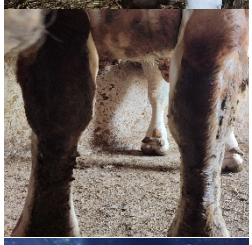
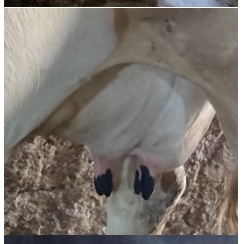
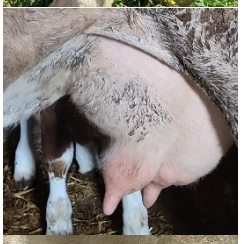
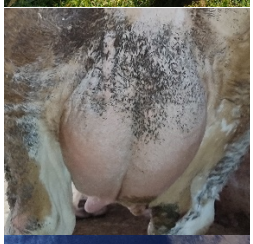
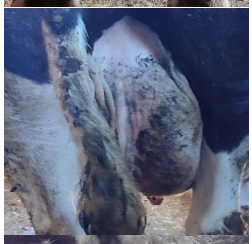
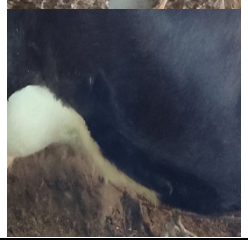
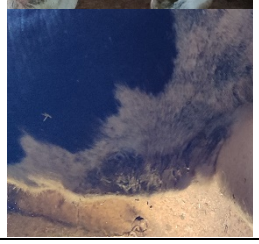
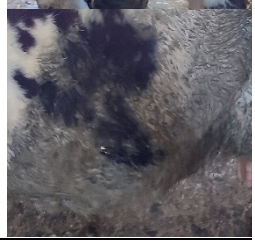
Ethical approval was also obtained from the Harran University Animal Experiments Local Ethics Committee, with decision number 2024/003/01-12 and ethics committee permission dated 16 May 2024, numbered 333691.

This study was conducted on 10 small family dairy farms. The number of animals varied between 2-15 within the borders of Şanlıurfa province. Data records were taken from 80 dairy cows in the study group, including 42 Holstein, 7 Holstein cross, 23 Simmental, 5 Simmental cross, and 3 Brown Swiss cows The ages of the cows ranged from 2 to 9 years. But the majority were between the ages of cows 3 and 5. Lactation periods ranged from 1 month to 12 months. The bedding materials of the barn were recorded according to the type of dairy farm where the animals were kept. Barn types: There were 3 different barn types: Free Stall, Tied Stall, and Semi-open. The bedding materials in the barns were: 1- Concrete bedding, 2- Garden soil, and concrete barn floor and 3- Rubber bedding. There were 3 different bedding bedding. In these family-type dairy farms, there was no application of cleaning the udder before and after milking. All farms were milking twice daily, in the morning (08:00 am) and evening (15:00 pm). After the above information of each animal was taken and recorded in the data notebook, the HS score of each region was given and recorded according to the Hygiene Score in Table 1. Photos were taken of animals on the farm (Fregonesi and Leaver 2001; Schreiner and Ruegg, 2003). The hygiene score is based on the cleanliness of the areas, ranging from the cleanest to the dirtiest. Scores were given as 1 point for the cleanest region and 4 points for the dirtiest region. . TheA sample of milk from each teat was transferred to a California Mastitis Test (Schalm and Noorlander, 1957) container, and CMT (KERBL, Germany) solution was added. The results were then interpreted. The results are scored in 4 different categories: No clotting and color change; negative (-), Mild clotting color change (+), Marked clotting and clumping (++), Immediate and severe clotting, extreme color change (+++).

Statistical Analysis: The hygiene score and Subclinical mastitis data were analyzed using the Kruskal-Wallis test, as they were not normally distributed according to the Shapiro-Wilk test. Additionally, the Pearson Correlation test was applied to show whether there was a relationship between the Hygiene score and Subclinical mastitis data. In addition, group comparisons were analyzed using the Kruskal-Wallis test.

Significance levels were considered $P<0,05$. All data were analyzed. All data were analyzed using the SPSS (version 28.0) statistical program.

Table 1. Hygiene score definition.

	Hygiene Score 1	Hygiene Score 2	Hygiene Score 3	Hygiene Score 4
Rear				
Thigh				
Leg				
Udder				
Belly				

Results

Since the hygiene score differs for each animal's HS region, each region is scored differently (Table 2). CMT results were negative in 50 animals. CMT was positive in a single mammary lobe in 18 animals (+,++,+++). In 10 animals, CMT was positive in two mammary lobes. In one animal, three mammary lobes were positive. Only one animal, all mammary lobes, was positive.

Table 2. Hygiene Score (HS) Points by number of animals

	HS 1	HS 2	HS 3	HS 4
Rear	55	20	4	1
Thigh	25	31	20	4
Leg	13	52	13	2
Udder	57	18	3	2
Belly	51	17	8	4

When the bedding materials in the barn were compared with the hygiene score; It was found that there was a significant relationship between the bedding material of the legs and the belly, respectively [χ^2(2, N=80)=6.60, P=0,037], [χ^2(2, N=80)=7.42, P=0,025]. Apart from these significant relationships, no significant relationship was found between bedding materials and hygiene scores (P>0,05). It was found that there was a significant difference between the hygiene score on the legs and the bedding materials made of concrete garden soil and barn concrete. While the average leg hygiene score of the animals in the barn with concrete bedding material is 2.4, the average leg hygiene score of the animals in the barn with garden soil and barn concrete bedding material is 1.4 (U=87.500; P=0,019). It was found that there was a significant difference between the hygiene score of the belly area and the bedding material rubber bedding and garden soil and barn concrete bedding materials. While the average belly hygiene score of the animals in the barn with rubber bedding is 2.6, the average leg hygiene score of the animals in the barn with garden soil and barn concrete material is 1.5 (U=100.000; P=0.010).

There was no relationship between the hygiene score and the CMT test result in any mammary lobe (P>0,05) (Table 2). It was found that there was a medium positive relationship between the rear and the leg in the hygiene score [*r*(80)=0,487, P<0,01)]. In the hygiene score, it was found that there was a low positive relationship between the rear and the feet (*r*(80)=0.343, P<0,01), udder (*r*(80)=0.269, P<0,05) and belly (*r*(80)=0.328, P<0,01). In the hygiene score, it was found that there was a medium positive relationship between the thigh and the leg (*r*(80)=0.548, P<0,01), udder (*r*(80)=0.505, P<0,01), and belly (*r*(80)=0.553, P<0,01).

It was found that there was a medium positive relationship between the leg and the udder and belly in the hygiene score [*r*(80)=0.411, P<0,01), *r*(80)=0.462, P<0,01)]. It was found that there was a high positive relationship between udder and belly in the hygiene score [*r*(80)=0.665, P<0,01)].

According to the CMT result, it was found that there was a medium positive relationship between the right front udder lobe, the left front udder lobe, and the right rear udder lobe [*r*(80)=0.309, P<0,01), *r*(80)=0.481, P<0,01)]. According to the CMT result, it was found that there was a medium positive relationship between the left front udder lobe and the right rear udder lobe [*r*(80)=0.424, P<0,01)] (Table 3).

No significant relationship existed between the body hygiene score and animals with positive CMT test results in any mammary lobe (P>0,05).

Table 3. Correlation of Hygiene Score and California Mastitis Test.

	HS-R	HS-T	HS-L	HS-U	HS-B	CMT-RFML	CMT- LFML	CMT- RRML	CMT- LRML
HS-R		0,487**	0,343**	0,269*	0,328**	0,056	0,036	0,020	0,032
HS-T			0,548**	0,505**	0,553**	0,102	0,139	0,087	-0,106
HS-L				0,411**	0,462**	0,161	0,074	0,164	-0,119
HS-U					0,665**	0,000	0,076	-0,054	0,036
HS-B						0,140	0,050	0,096	-0,026
CMT-RFML							0,309**	0,481**	0,077
CMT- LFML								0,424**	0,179
CMT- RRML									0,076
CMT- LRML									

HS-R: Hygiene Score Rear, HS-T: Hygiene Score Thigh, HS-L: Hygiene Score Leg, HS U: Hygiene Score Udder, HS-B: Hygiene Score Belly, CMT: California Mastitis Test, RFML: Right Front Mammary Lobe, LAML: Left Front Mammary Lobe, RRML: Right Rear Mammary Lobe, LRML: Left Rear Mammary Lobe ** P<0,01 , * P<0,05.

There is no significant relationship found between animals with positive CMT test results in any mammary lobe in terms of barn type, pad material, breeds, age, lactation periods, lactation duration, and milk yield, respectively (P>0,05).

When the general average hygiene score of each animal was examined according to the CMT result in any udder lobe, it was found that the number of animals with (-) CMT results was higher and the number of animals with low hygiene scores was higher. Animals with CMT results of (+), (++), and (+++) have a high average hygiene score. However, no statistically significant relationship was found between them (Figure 1).

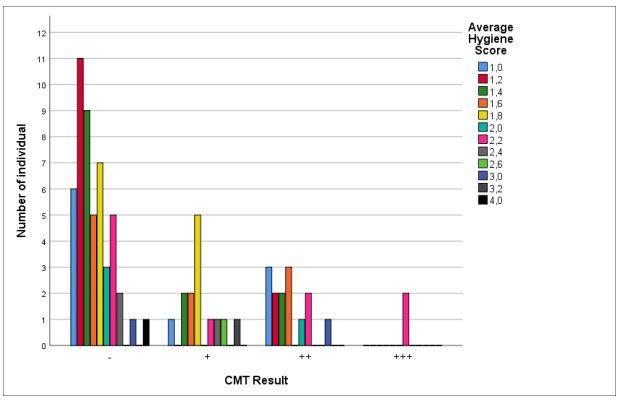


Figure 1. Each animal's average Hygiene Score with relation to the animal's average Hygiene Score in relation to CMT results.

Discussion and Conclusion

According to our results, the hygiene score of each region was different from the others. In addition, hygiene scores in interconnected body parts affect each other positively. In the results reported by Schreiner and Ruegg (2003), it was found that as the udder thigh score, which is considered the hygiene score, increases, the rate of subclinical mastitis increases, and the risk of developing mastitis increases. For example, there is a high-level correlation between the udder area and the abdominal area hygiene score. In addition, a relationship was found between the CMT test and subclinical mastitis between udder lobes in the same direction. We found that the Hygiene score in barns with concrete backing was higher than in barns with other bedding materials. In this study, it showed that the Hygiene scores are also high in concrete-based barns, since the animals are kept tied and always sleep in the same place. It has been reported that the hygiene region is effective with the type of bedding and bedding material in the barn. (Koçyiğit and Tüzemen, 2015). It is supported by our findings. It has been reported that there is a highly significant relationship between udder and foot hygiene in the hygiene score (Schreiner and Ruegg, 2003). This situation is supported by our findings. It is also reported that foot and udder hygiene scores increase in cows that lie in poor barn conditions for longer periods of time (DeVries et al., 2012).

We did not get the results we expected in our findings. We expected the CMT positivity level to be very high in

animals with high hygiene scores. However, since many factors have been shown to be among the causes of subclinical mastitis, making a judgment based only on hygiene will not yield the expected result. It has been reported that SCC (<200,000 µmL1) in milk is low under hygienic milking conditions, and SCC in milk under unhygienic milking conditions increases and increases the incidence of clinical mastitis (Emre et al., 2011). Additionally, are known a high correlation between CMT and SCC. The relationship between subclinical mastitis and the hygiene score with CMT test is thought to be insufficient. In addition to the relationship between the hygiene score and CMT, performing the SCC test will give stronger results. It was understood that the presence of subclinical mastitis by the CMT test was not sufficient to explain the relationship between the hygiene score. We would like to point out that other tests are needed to detect subclinical mastitis.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Authors Contributions

Motivation / Concept: AU
Design: AU, AO
Control/Supervision: AU, AO
Data Collection and/or Processing: AU
Analysis and / or Interpretation: AO
Literature Review: AO
Writing the Article: AO
Critical Review: AU

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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