Meta-analysis and meta-regression of subclinical mastitis prevalences in dairy cattle in Türkiye

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ABSTRACT

In this study, it was aimed to determine the prevalence of subclinical mastitis obtained from 38 studies conducted in Türkiye between 1988 and 2019 by meta-analysis method and to calculate common prevalence. The estimated prevalence of subclinical mastitis in the studies were evaluated separately as cow-based (11182 cows in 37 studies) and udder quarter-based (48990 udder quarters in 33 studies). According to the results, the common prevalence of subclinical mastitis was calculated as 0.48 (95% CI: 0.41-0.56) in cow-based studies and 0.32 (95% CI: 0.26-0.37) in quarter based-based studies. As a result of this study, the results of individual studies on the prevalence of subclinical mastitis in dairy cows in Türkiye were combined with the meta-analysis method and a more precise estimate of the prevalence was obtained.

Introduction

Mastitis is an infection that occurs in the mammary gland as a result of infectious agents such as bacteria, yeast, fungi, and viruses entering the body through the teat canal. The form of mastitis in which milk or mammary gland abnormalities or secondary clinical signs can be observed is defined as clinical mastitis; and the form that can be diagnosed by using different test methods to identify the inflammatory cells formed by the glandular tissue but cannot otherwise be detected is defined as subclinical mastitis (6, 9, 18).

Subclinical mastitis is a type of mastitis that has a high risk of contamination in the herd and can turn into clinical mastitis, if not treated, causing deterioration in milk structure and decrease in milk yield (9). It has been reported that subclinical mastitis causes more economic loss than clinical mastitis in the dairy industry due to the difficulty of its detection. The economic loss due to subclinical mastitis in Türkiye has been estimated to be approximately 8 trillion dollars per year, whereas the amount allocated for maintaining mammary health has been determined to be 33 dollars per animal (6, 9, 14).

Sublinical mastitis and its economic losses can be avoided by following a versatile mastitis control plan in the herds (6, 18). Subclinical mastitis is one of the most important problems of the dairy industry and is still up to date. Since many studies have been conducted both in the world and in Türkiye to estimate the prevalence of subclinical mastitis and to determine the factors affecting it, systematic review or meta-analysis studies are needed on this subject.

Due to variations in the achieved results, the results should be statistically interprited according to breed, herd size and geographic properties (1, 13).

In this study, it was aimed to evaluate the prevalence of subclinical mastitis in dairy cows in Türkiye by meta-analysis and to calculate the common prevalence as well as to determine the effects of breed, geographical region, the years of the studies, and herd size on subclinical mastitis by meta-regression analysis.

Materials and Methods

Thirty-8 studies, which were about the prevalence of subclinical mastitis in Türkiye and published between 1988 and 2019 were evaluated in this study. In these studies, cows and udder quarters were used as materials to determine the prevalence of subclinical mastitis. For this reason, meta-analyses in the study were conducted separately as cow-based and udder quarter-based. The characteristics of these studies were given in Table 1.

Table 1. Characteristics of studies included in the meta-analysis of subclinical mastitis prevalences in Türkiye.

Rank No	Authors	Year	Province	Breed	Base on *
1	**Alaçam E, Tekeli T, Erganiş O, et al** (1989): *The Diagnosis, Isolation of Etiological Agents and Antibiotic Susceptibility Test Results in Cows and Buffalos Suffering from Subclinical Mastitis*. Eurasian J Vet Sci **5**, 91–101.	1989	Afyonkarahisar	Brown Swiss and Crossbreed	1
2	**Nizamlıoğlu M, Kalaycıoğlu L, Dinç DA, et al** (1992): *Determination of N-acetyl B-D glucosaminidase enzyme activity in bovine milk for the early diagnosis of subclinical mastitis*. S Ü Vet Fak Derg **8**, 60–63.	1992	Konya	Holstein and Crossbreed	1
3	**Gürtürk K, Boynukara B, Ekin İH, et al** (1998*): A study on the etiology of subclinical mastitis in dairy cows in and around Van*. Van Vet J **9**: 1–4.	1998	Van	Unspecified Breeds	2
4	**Vural MR, Esendal Ö, İzgür H, et al** (1999): *Subclinical mastitis cases in primiparous holstein cows part 2. Intramammary infections during first lactation*. Veterinary Journal of Ankara University **46**, 287 - 298.	1999	Ankara	Holstein and Crossbreed	1, 2
5	**Yüksel H** (1999): *Pathologic investigations on mastitis in cows slaughtered at the Elazığ Elet abattoir*. PhD thesis, Fırat University Institute of Health Sciences, Elazığ.	1999	Elazığ	Indigenous Breeds and Crossbreed	2
6	**Ak S** (2000): *Bacterial agents cause contagious and environmental bovine mastitis in Trakya district and their susceptibility to antibiotics*. J Fac Vet Med Univ Istanbul **26**, 353–365.	2000	-	Unspecified Breeds	1, 2
7	**Şeker İ, Rişvanlı A, Kul S, et al** (2000): *Relationships Between CMT Scores and Udder Traits and Milk Yield in Brown-Swiss Cows*. Lalahan Hay Araşt Enst Derg **40**, 29–38.	2000	Malatya	Brown Swiss and Crossbreed	2
8	**Uzmay C, Kaya A, Akba Y** (2001): *Studies on Prevalence of Mastitis and Factors Affecting Prevalence in Herds of Izmir Holstein Breeders Association. 2. Relationships Between Managerial Practices and Subclinical Mastitis*. Ege Üniv. Ziraat Fak. Derg **38**, 71-78.	2001	İzmir	Unspecified Breeds	1, 2
9	**Rişvanlı A** (2001): *Clinic and subclinic incidence and isolation, antibiotic sensitivity of the microorganisms caused mastitis in dairy cows in Elazığ district*. PhD thesis, Fırat University Institute of Health Sciences, Elazığ.	2001	Elazığ	Holstein and Crossbreed, Brown Swiss and Crossbreed, Indigenous Breeds and Crossbreed, Other Breeds	1, 2
10	**Beytut E, Aydın F, Özcan K, et al** (2002): *Pathological and Bacteriological Investigations on Bovine Mastitis in Kars Region and Its Surround*. Kafkas Üniv Vet Fak Derg **8**, 111–122.	2002	Kars	Unspecified Breeds	1
11	**Kireçci E, Çolak A** (2002*): Methicillin Resistance in Staphylococci Strains Isolated from Dairy Cows with Subclinical Mastitis the Onset of the Dry Period*. Kafkas Üniv Vet Fak Derg 8, 98–100.	2002	Erzurum	Other Breeds	1, 2
12	**Uzmay C, Kaya I, Akbaş Y, et al** (2003): *Effects of Udder and Teat Morphology, Parity and Lactation Stage on Subclinical Mastitis in Holstein Cows*. Turkish J Vet Anim Sci **27**, 695-701.	2003	İzmir	Unspecified Breeds	1
13	**Sabuncuoğlu N, Çolak A, Akbulut Ö, et al** (2003): *Relationships Between CMT Scores and Some Milk Yield Traits in Holstein Friesian and Brown Swiss Cows.* Atatürk Univ. J. of Agricultural Faculty **34**, 139–143.	2003	Erzurum	Brown Swiss and Crossbreed, Holstein and Crossbreed	2
14	**Ergün Y, Aslantaş Ö, Doğruer G, et al** (2004): *Epidemiology of Subclinical Mastit is in Family Size Dairy Farms in Hatay Region*. Vet Bil Derg **20**, 25–28.	2004	Hatay	Holstein and Crossbreed	1
15	**Gülcü HB, Ertaş HB** (2004): *Bacteriological Investigation of Udder Lobes of Cows with Mastitis Slaughtered in the Elazığ Region*. Turk J Vet Anim Sci **28**, 91–94.	2004	Elazığ	Indigenous Breeds and Crossbreed	1, 2
16	**Abay M, Bekyürek T** (2006): *The Compare of Efficiency of Cefquinome and Amoxycillin+Clavulonic Acide Treatment on Subclinical Staphylococcus Aureus Mastitis in Lactating Dairy Cows*. JHSM **15**, 189–193.	2006	Sivas	Holstein and Crossbreed	1
17	**Musal B, İzgür İH** (2006): *The efficacy of intramammary, systemic and combined antibiotics administered during dry off in cows with subclinical mastitis*. Ankara Univ Vet Fak Derg **53**, 175–178.	2006	Eskişehir	Brown Swiss and Crossbreed	1, 2

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Rank No	Authors	Year	Province	Breed	Base on *
18	**Çoban Ö, Tüzemen N** (2007): *Risk Factors For Subclinical Mastitis In Holstein Friesian and Brown Swiss Cows*. Uludag Univ J Fac Vet Med **26**, 27–31.	2007	Erzurum	Other Breeds	2
19	**Baştan A, Kaçar C, Acar DB, et al** (2008): *Investigation of the incidence and diagnosis of subclinical mastitis in early lactation period cows*. Turk J Vet Anim Sci **32**, 119–121.	2008	-	Unspecified Breeds	2
20	**Tel OY, Keskin O, Zonturlu AK, et al** (2009): *Subclinical Mastitis Prevalance and Determination of The Antibiotics Susceptibility in Sanliurfa Region*. F.U. Vet.J.Health.Sci **23**, 101–106.	2009	Şanlıurfa	Unspecified Breeds	1, 2
21	**Türkyılmaz S, Yıldız Ö, Oryaşın E, et al** (2010): *Molecular identification of bacteria isolated from dairy herds with mastitis*. Kafkas Üniv Vet Fak Derg **16**, 1025–1032.	2010	-	Unspecified Breeds	1
22	**Macun HC, Pir Yağcı İ, Ünal N, et al** (2011): *Agent Isolation and Antibiotic Resistance in Dairy Cows with Subclinical Mastitis in Kırıkkale*. J Fac Vet Med Univ Erciyes **8**, 83–89.	2011	Kırıkkale	Unspecified Breeds	1
23	**Bardakcioglu HE, Sekkin S, Oral Toplu HD** (2011): *Relationship between some teat and body measurements of Holstein cows and sub-clinical mastitis and milk yield*. J Anim Vet Adv **10**, 1735–1737.	2011	Aydın	Holstein and Crossbreed	1, 2
24	**Yeşilmen S, Özyurtlu N, Bademkıran S** (2012): *The Isolation of Subclinical Mastitis Agents and Determination of the Sensitive Antibiotics in Dairy Cows in Diyarbakır Province*. Dicle Üniv Vet Fak Derg **1**, 24–29.	2012	Diyarbakır	Unspecified Breeds	1
25	**Acar G, Yılmaz E, Solmaz H, et al** (2012): *Isolation of Streptococcal Agents from Cattle with Subclinical Mastitis in Hatay Region and Detection of their Susceptibilities against some Antibiotics*. AVKAE Derg **2**, 1-5	2012	Hatay	Unspecified Breeds	1, 2
26	**Çokal Y, Konuş R** (2012): *Isolation of Aerobic Bacteria From Cow Milks With Subclinical Mastitis*. BAUN Health Sci J **1**, 65–69.	2012	Balıkesir	Unspecified Breeds	1, 2
27	**Koçyiğit R** (2012): *The determination of subclinical mastitis incidence in dairy cows in Bolu Mudurnu region*. MSc thesis, Afyon Kocatepe University Institute of Health Sciences, Afyonkarahisar.	2012	Bolu	Other Breeds	1, 2
28	**Ayanoğlu K** (2012): *Investigation of the Effect of Pregnancy Rate of Mastitis That Formed in Cows During Early Pregnancy*. PhD thesis, Selçuk University	2012	Isparta	Holstein and Crossbreed	1, 2
29	**Kaygısız A, Karnak İ** (2012): *Evaluation of Somatic Cell Count in Raw Milk Samples Collected from Dairy Farms in Kahramanmaras Province for EU Norms and Subclinical Mastitis*. KSU J Nat Sci **15**, 9-15.	2012	Kahramanmaraş	Holstein and Crossbreed	2
30	**Ikiz S, Başaran B, Bingöl EB, et al** (2013): *Presence and antibiotic susceptibility patterns of contagious mastitis agents (staphylococcus aureus and streptococcus agalactiae) isolated from milks of dairy cows with subclinical mastitis*. Turk J Vet Anim Sci **37**, 569–574.	2013	-	Unspecified Breeds	1
31	**Özdemir S, Kaymaz M** (2013): *Comparison of Diagnostic Methods and Incidence of Subclinical Mastitis on Local Breeds*. Atatürk University J Vet Sci **8**, 71–79.	2013	Sivas	Unspecified Breeds	2
32	**Büyükcangaz E, Mat B, Ahmed MKAA** (2012): *Microbiological Analysis and Antimicrobial Resistance Pattern of the Isolates Derived From Dairy Cattle With Subclinical Mastitis*. Uludag Univ J Fac Vet Med **31**, 35–44.	2013	Bursa	Holstein and Crossbreed	2
33	**Baştan A, Salar S, Cengiz M, et al** (2015): *The prediction of the prevalence and risk factors for subclinical heifer mastitis in Turkish dairy farms*. Turk J Vet Anim Sci **39**, 682–687.	2015	-	Unspecified Breeds	1
34	**Gezgen C** (2015): *Investigation of methicillin resistance and Panton-Valentine leukocidin in Staphylococci isolated from bovine mastitis*. MSc thesis, Afyon Kocatepe University Institute of Health Sciences, Afyonkarahisar.	2015	İzmir	Unspecified Breeds	1, 2
35	**Dalgıç D, Sarıbay MK** (2015): *Distribution of Lesions Occurred in Teat Skin and Their Effects on Mastitis in Cows*. F.U. Vet.J.Health.Sci **29**, 111–117.	2015	Hatay	Holstein and Crossbreed	2
36	**Akdağ F, Gürler H, Teke B, et al** (2017): *The Effect of the Scores and Various Assessments of the Scores for CMT on Milk Yield, Milk Composition and the Diagnosis of Subclinical Mastitis in Jersey Cows*. J Fac Vet Med Istanbul Univ **43**, 44–51.	2016	Samsun	Other Breeds	1
37	**Tepeli SÖ, Zorba NN** (2017): *Some Properties of Raw Milk Produced in Çanakkale (Yenice) City and The Incidence of Subclinical (Hidden) Mastitis*. TUJNS **18**, 41–47.	2017	Çanakkale	Unspecified Breeds	1
38	**Saydan M, Kalkan C** (2017): *Prevalence of Subclinical Mastitis in Dairy Cattle in Malatya Arguvan District*. F.U. Vet.J.Health.Sci **31**, 193–200.	2017	Malatya	Holstein and Crossbreed, Brown Swiss and Crossbreed, Other Breeds	1, 2

*: 1=Cow-based Prevalence, 2=Quarter-based Prevalence.

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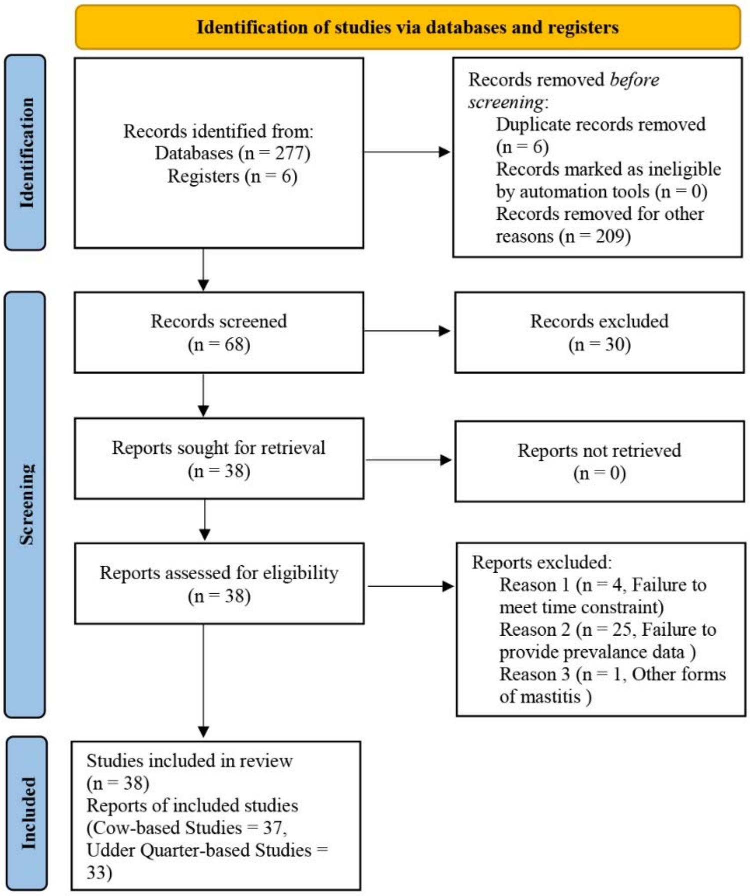

Figure 1. Flow diagram of prevalences of subclinical mastitis in dairy cows (16).

The literature search for the studies to be included in the meta-analysis was carried out between 06.07.2017 and 03.05.2020. The keywords were chosen as 'Subclinical mastitis', 'Dairy cattle', and 'Prevalence', and the literature search was made using Google Scholar, PubMed, ScienceDirect, and Scopus electronic databases. The inclusion criteria for the studies used in the meta-analysis were determined as "have been done in Türkiye and to have the calculated or calculable prevalence of subclinical mastitis in dairy cow breeds". Studies to be included in meta-analyses were determined according to the PRISMA 2020 checklist and the flowchart was given in Figure 1 (17). Meeting the inclusion criteria as a result of literature search, subclinical mastitis prevalence data from 11182 dairy cows in 37 cow-based studies and 48990 udder quarters from 33 udder-quarters-based studies were used in the meta-analyses. In the study samples, Begg and Mazumdar rank correlation test and Egger's linear regression test were performed to determine publication biases, and the funnel plots were drawn. One Study Removed method was used to determine the effect of individual studies on the common prevalence.

Der Simonian-Laird method was used to determine the heterogeneity between studies. In order to determine the sources of heterogeneity, subgroups were formed according to the categories of region, breed, year groups, and herd size covariates, and meta-regression analyses of subclinical mastitis prevalence were performed for each group. Subgroups with fewer than three studies were not included in the analysis.

For the subgroup analyses conducted separately for cow and udder quarter-based studies, the subgroups were formed as 5 geographical regions in Türkiye (Mediterranean Region, Eastern Anatolia Region, Aegean Region, Central Anatolia Region, and Marmara Region), cow breeds (Holstein and its crossbreeds, Swiss Brown and its crossbreeds, the local breed and its crossbreeds, other breeds, and unspecified breeds), year groups (1988-1999, 2000-2009, 2010-2019) and herd sizes (small ($n<100$), medium ($100\leq n<300$), large ($300\leq n$)).

In the applied meta-regression analyzes, method of moments was preferred for the calculation of the model coefficients. Comprehensive Meta-Analysis (CMA) and R 4.1.0 (www.r-project.org) softwares were used in the application of meta-analyses. In R software, "meta", "metaphor" and "tidyverse" packages were used. In order to test the hypothesis that the prevalence was not different from 0.5 in the analysis, the significance level was determined as $P<0.05$, while the significance level for the significance controls of Cochran's Q heterogeneity statistics was taken as $P<0.10$.

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Results

Cow and udder quarter-based meta-analyses were conducted regarding the prevalence of subclinical mastitis throughout Türkiye, and according to the results of Der Simonian-Laird method used to determine the heterogeneity among the studies, high heterogeneity was detected between both cow-based and udder quarter-based studies (Cochrane's Q=4918.44, df=36, I^2=99.27, P<0.001; Cochrane's Q=11907.87, df=32, I^2=99.73, respectively) (Table 2). Thus, the random effect model was used to calculate the effect sizes in the analyses. According to the results of the random effects model, the common prevalence of subclinical mastitis in Türkiye was calculated as 0.48 (95% CI: 0.41-0.56) in cow-based studies and 0.32 (95% CI: 0.26-0.37) in udder quarter-based studies (Table 2).

According to the results of One Study Removed method, which was used to determine the effects of individual studies on the prevalence, it was observed that the pooled prevalence estimation did not change when the studies were removed one by one from the analysis. The forest plots of the studies were given in Figure 2.

According to Begg and Mazumdar rank correlation test and Egger's linear regression test which was used to detect publication bias in the study samples, there was no publication bias in the study samples (P>0.05) (Table 3). In addition, no asymmetry was observed in the funnel plots (Figure 3).

For cow and udder quarter-based studies, separate subgroup analyses were made with the variables of 5 geographical regions in Türkiye, cow breeds, year groups, and herd sizes. This subgroup analyses were made according to geographical regions in Türkiye. The Southeastern Anatolia Region and the Black Sea Region, where there were not enough studies to form a group, were excluded from the analysis, and the remaining 5 regions were compared. It was determined that the prevalence of subclinical mastitis varies according to regions in both cow and udder quarter-based studies (P=0.071, P=0.008, respectively). In the cow-based studies, the highest prevalence of subclinical mastitis was calculated in the Marmara Region (0.68), and the lowest prevalence was calculated in the Aegean Region (0.33); in the udder quarter-based studies the highest prevalence was calculated in the Central Anatolian Region (0.50), and the lowest prevalence was calculated in the Marmara Region (0.14) (Table 4).

It was determined that the prevalence of subclinical mastitis did not show a statistically significant variation in the subgroup analyses according to cow breeds (P=0.687) (Table 5).

In the subgroup analyses performed according to the years of the studies, it was determined that the prevalence of subclinical mastitis showed a statistically significant variation (P=0.053). Accordingly, the highest prevalence values in both the cow-based studies and the udder quarter-based studies were calculated between 2010 and 2019 (0.54 and 0.32, respectively), and the lowest prevalence values were calculated between 1988 and 1999 (0.17 and 0.12, respectively) (Table 6).

In the subgroup analyses performed according to the herd's size used in the studies, the prevalence of subclinical mastitis did not show a statistically significant variation in the cow-based studies (P=0.207), while it showed a significant variation in udder quarter-based studies (P<0.001). Accordingly, the highest prevalence value in udder quarter-based studies was observed in small-scale herds (0.42) (Table 7).

In this study, meta-regression analysis was performed in order to theoretically calculate the sources of heterogeneity among the studies included in the meta-analysis. For this purpose, multivariate meta-regression models were created for both the cow and the udder quarter-based studies.

The model with the highest R^2 analog value in the multivariate meta-regression analysis created for cow-based and udder quarter-based studies is given in Table 8.

According to the model test for the cow-based studies, there was a significant difference in disease prevalence between the subgroups of the year group and herd size variables (Q=9.42, df=4, P=0.052). This model explains 28% of the variance in the true effects (R^2 analog=0.28). In this model, the effects of year groups and herd size were found to be significant. When 1988-1999 subgroup was taken as the reference, the prevalence of subclinical mastitis was calculated to be 1.22 times higher in 2000-2009; and 1.63 times higher in 2010-2019 (P<0.05). When the large-scale herds were taken the reference, the prevalence of subclinical mastitis was calculated to be 0.40 times higher in medium-sized herds and 0.68 times higher in small-scale herds (P>0.05). For udder quarter-based studies, there was a significant difference in disease prevalence among all subgroups of the region, year group, and herd size variables according to the model test (Q=33.16, df=8, P<0.001). This model explains 30% of the variance in the true effects (R^2 analog=0.30). According to the model, when the Marmara Region was taken as the reference, the prevalence of subclinical mastitis was 1.57 times higher in the Aegean Region and 1.73 times higher in the Central Anatolia Region (P<0.05).

When 1988-1999 subgroup was taken as the reference, the prevalence of subclinical mastitis was calculated to be 1.08 times higher in 2000-2009, and 1.38 times higher in 2010-2019 (P<0.05). When large-scale herds are taken as the reference, the prevalence of subclinical mastitis was calculated to be 0.50 times higher in medium-sized herds and 0.97 times higher in small-scale herds (P>0.05, P<0.05, respectively).

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Table 2. Cow-based and udder quarter-based pooled subclinical mastitis prevalences across Türkiye.

Statistics	Cow-based (n=37)	Quarter-based (n=33)
Total Numbers	11182	48990
No of Subclinical Mastitis (+)	3596	10310
Simple Ratio	0.322	0.210
Pooled Prevalence and 95% C.I.	**0.48 (0.41-0.56)**	**0.32 (0.26-0.37)**
P Value	<0.001	<0.001
Heterogeneity Test		
Cochran's Q	4918.44	11907.87
df (Cochran's Q)	36	32
τ^2	0.058	0.025
I^2 (%)	99.27	99.73
P Value (Cochran's Q)	**<0.001**	**<0.001**

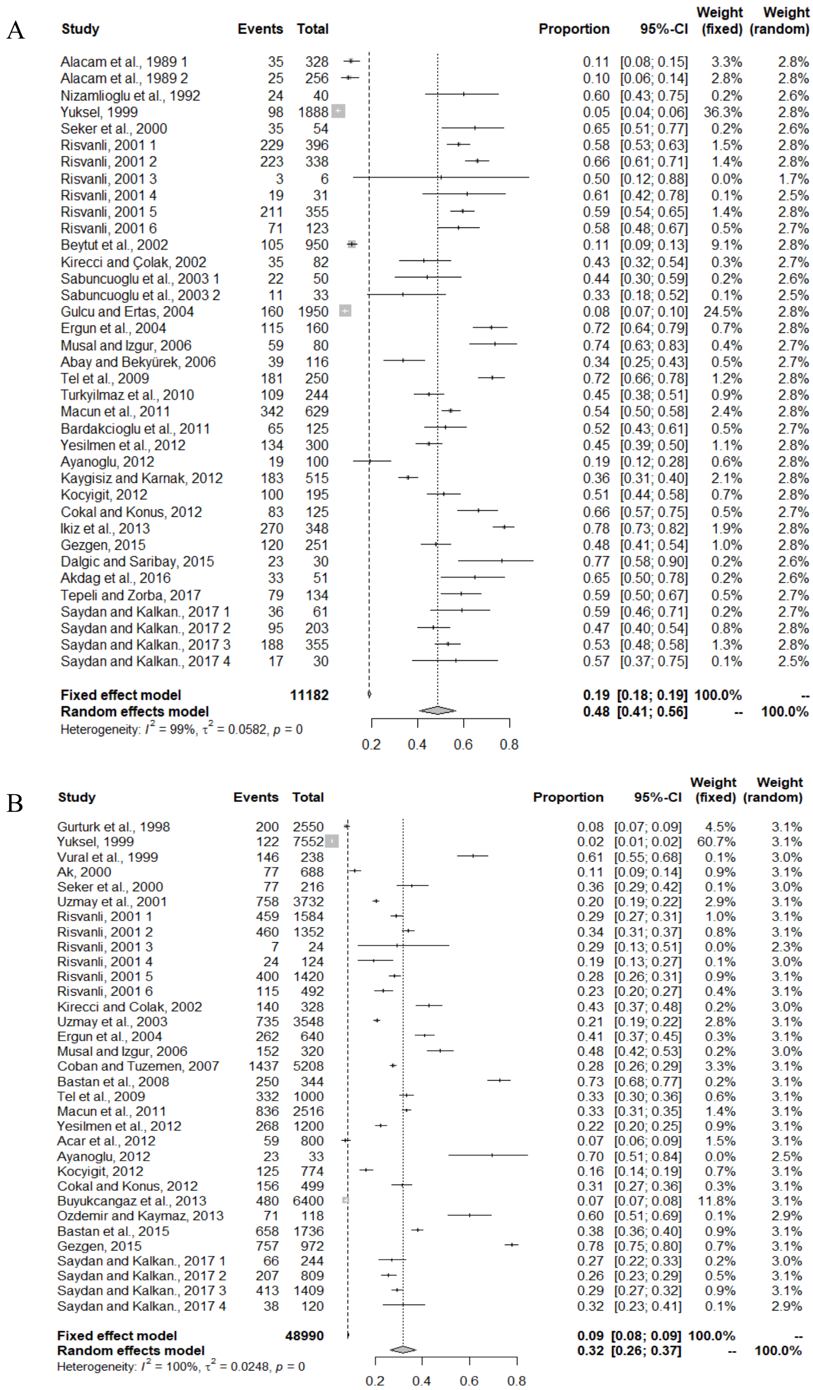

Figure 2. A) Forest plot of cow-based meta-analysis **B)** Forest plot of udder quarter-based meta-analysis.

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Table 3. Publication bias tests of cow-based and udder quarter-based study samples.

	Begg and Mazumdar Rank Correlation Test		Egger's Regression Test	
	Cow-Based	**Quarter-Based**	**Cow-Based**	**Quarter-Based**
Intercept	-0.068	<0.001	5.064	2.318
t statistic	-	-	1.610	0.501
z statistic	0.586	<0.001	-	-
P Value	0.558	1.000	0.117	0.621

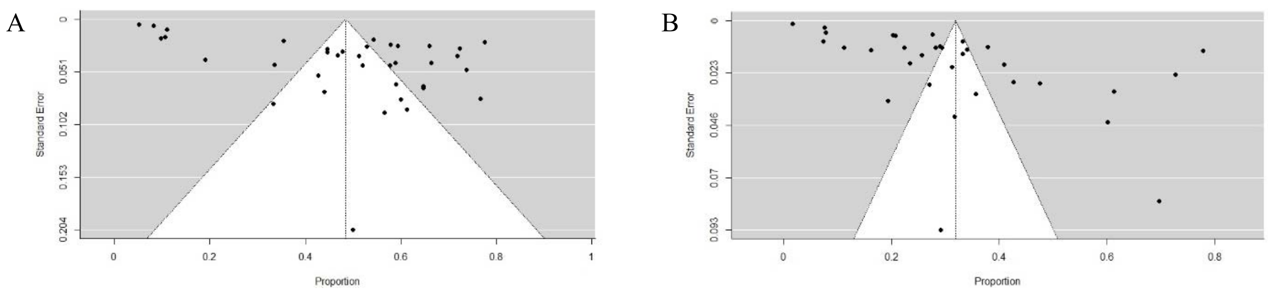

Figure 3. A) Cow-based funnel plot of the study sample on subclinical mastitis prevalences **B)** Udder quarter-based funnel plot of the study sample on subclinical mastitis prevalences across Türkiye.

Table 4. Results of subgroup analysis of cow-based and quarter-based subclinical mastitis prevalences in Türkiye by region.

	Regions				
	The Mediterranean region (n=4)	**The Eastern Anatolia Region (n=18)**	**The Aegean Region (n=3)**	**The Central Anatolia Region (n=4)**	**The Marmara Region (n=3)**
Cow-Based (n=32)					
Number of Cows	805	7205	704	865	607
Number of Cows with Subclinical Mastitis (+)	340	1692	202	464	432
Simple Proportion	0.42	0.24	0.29	0.54	0.71
Pooled Prevalence and 95% CI	0.50 (0.26-0.74)	0.42 (0.28-0.58)	0.33 (0.12-0.64)	0.55 (0.40-0.69)	0.68 (0.56-0.79)
P Value	0.988	0.338	0.286	0.491	**0.006**
Heterogeneuity Test					
Cochran's Q	92.05	1584.61	103.31	30.64	17.72
df (Cochran's Q)	3	17	2	3	2
τ^2	1.085	1.886	1.296	0.327	0.208
I^2	96.741	98.927	98.064	90.208	88.710
P Value (Cochran's Q)	<0.001	<0.001	<0.001	<0.001	<0.001
Between Regions					
Cochran's Q =8.635, df=4, p= 0.071					
Udder Quarter-Based (n=29)	**The Mediterranean region (n=3)**	**The Eastern Anatolia Region (n=16)**	**The Aegean Region (n=3)**	**The Central Anatolia Region (n=4)**	**The Marmara Region (n=3)**
Number of Udder Quarter	1473	24632	8252	3192	7587
Number of Udder Quarter with Subclinical Mastitis (+)	344	4433	2250	1205	713
Simple Proportion	0.23	0.18	0.27	0.38	0.09
Pooled Prevalence and 95% CI	0.33 (0.08-0.73)	0.23 (0.17-0.30)	0.38 (0.15-0.68)	0.50 (0.35-0.65)	0.14 (0.05-0.33)
P Value	0.417	**<0.001**	0.449	0.982	**0.001**
Heterogeneity Test					
Cochran's Q	210.103	1615.283	1002.477	110.385	257.516
df (Cochran's Q)	2	15	2	3	2
τ^2	2.270	0.584	1.230	0.387	0.928
I^2	99.048	99.071	99.800	97.282	99.223
P Value (Cochran's Q)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Between Regions					
Cochran's Q =13.802, df=4, P= 0.008					

df= degree of freedom, C.I.: Confidence Interval, SE: Standard Error, I^2: Ratio of variance in observed effects to variance in true effects rather than sampling error, τ^2: Variance in true effect sizes.

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Table 5. Subgroup analysis results of cow-based and quarter-based subclinical mastitis prevalences in Türkiye by breed.

	Breeds				
	Holstein and Crossbreed (n=10)	**Brown Swiss and Crossbreed (n=6)**	**Indigenous and Crossbreed (n=4)**	**Other Breeds (n=5)**	**Unspecified Breeds (n=7)**
Cow-Based (n=32)					
Number of Cows	1.518	1111	4316	504	2737
Number of Cows with Subclinical Mastitis (+)	738	475	540	262	1133
Simple Proportion	0.49	0.43	0.13	0.52	0.41
Pooled Prevalence and 95% CI	0.50 (0.38-0.62)	0.48 (0.28-0.68)	0.24 (0.06-0.62)	0.52 (0.47-0.57)	0.50 (0.31-0.70)
P Value	0.967	0.821	0.169	0.467	0.987
Heterogeneity Test					
Cochran's Q	161.986	171.267	701.100	4.279	511.783
df (Cochran's Q)	9	5	3	4	6
τ^2	0.572	1.058	2.823	0.005	1.226
I^2	94.444	97.081	99.572	6.518	98.828
P Value (Cochran's Q)	**<0.001**	**<0.001**	**<0.001**	0.370	**<0.001**
Between Breeds					
Cochran's Q = 2.263, df=4, P = 0.687					
Udder Quarter-Based (n=29)					
	Holstein and Crossbreed (n=6)	**Brown Swiss and Crossbreed (n=4)**	**Indigenous and Crossbreed (n=3)**	**Other Breeds (n=6)**	**Unspecified Breeds (n=10)**
Number of Udder Quarter	8907	2929	9464	7213	16623
Number of Udder Quarter with Subclinical Mastitis (+)	1437	895	637	2059	3917
Simple Proportion	0.16	0.31	0.07	0.29	0.24
Pooled Prevalence and 95% CI	0.36 (0.17-0.61)	0.34 (0.26-0.43)	0.11 (0.02-0.48)	0.30 (0.26-0.35)	0.25 (0.16-0.37)
P Value	0.282	<0.001	0.042	<0.001	<0.001
Heterogeneity Test					
Cochran's Q	1117.273	55.256	886.331	39.425	1697.403
df (Cochran's Q)	5	3	2	5	9
τ^2	1.590	0.137	3.113	0.052	0.797
I^2	99.552	94.571	99.774	87.318	99.470
P Value (Cochran's Q)	<0.001	<0.001	<0.001	<0.001	<0.001
Between Breeds					
Cochran's Q =3.252, df=4, P=0.517					

df= degree of freedom, C.I.: Confidence Interval, SE: Standard Error, I^2: Ratio of variance in observed effects to variance in true effects rather than sampling error, τ^2: Variance in true effect sizes.

Table 6. Subgroup analysis results of cow-based and quarter-based subclinical mastitis prevalences in Türkiye by years.

	Years		
	1988-1999 (n=3)	**2000-2009 (n=14)**	**2010-2019 (n=15)**
Cow-Based (n=32)			
Number of Cows	2256	4564	3366
Number of Cows with Subclinical Mastitis (+)	157	1222	1769
Simple Proportion	0.07	0.27	0.53
Pooled Prevalence and 95% CI	0.17 (0.05-0.47)	0.45 (0.28-0.63)	0.54 (0.47-0.62)
P Value	<0.001	<0.001	0.012
Heterogeneity Test			
Cochran's Q	100.652	1066.515	227.944
df (Cochran's Q)	2	13	14
τ^2	1.611	1.941	0.309
I^2	98.013	98.781	93.858
P Value (Cochran's Q)	<0.001	<0.001	<0.001
Between Years			
Cochran's Q =5.890, df=2, P=0.053			

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Quarter-Based (n=29)			
	1988-1999 (n=3)	**2000-2009 (n=12)**	**2010-2019 (n=14)**
Number of Udder Quarters	10340	13828	20968
Number of Udder Quarters with Subclinical Mastitis (+)	468	3404	5073
Simple Proportion	0.05	0.25	0.24
Pooled Prevalence and 95% CI	0.12 (0.02-0.53)	0.27 (0.23-0.33)	0.32 (0.23-0.43)
P Value	0.066	<0.001	0.002
Heterogeneity Test			
Cochran's Q	805.132	368.279	2215.519
df (Cochran's Q)	2	11	13
τ^2	3.687	0.171	0.804
I^2	99.752	97.013	99.413
P Value (Cochran's Q)	<0.001	<0.001	<0.001
Between Years			
Cochran's Q = 1.666, df=2, P= 0.435			

df= degree of freedom, C.I.: Confidence Interval, SE: Standard Error, I^2: Ratio of variance in observed effects to variance in true effects rather than sampling error, τ^2: Variance in true effect sizes.

Table 7. Subgroup analysis results of cow-based and quarter-based subclinical mastitis prevalences in Türkiye according to herd size.

	Herd Sizes		
	Small (n=12)	**Medium (n=9)**	**Large (n=11)**
Cow-Based (n=32)			
Number of Cows	597	1537	8052
Number of Cows with Subclinical Mastitis (+)	303	801	2044
Simple Proportion	0.51	0.52	0.25
Pooled Prevalence and 95% CI	0.53 (0.42-0.64)	0.53 (0.46-0.61)	0.34 (0.18-0.55)
P Value	0.58	0.37	0.12
Heterogeneity Test			
Cochran's Q	72.154	63.414	1875.242
df (Cochran's Q)	11	8	10
τ^2	0.528	0.173	1.988
I^2	84.755	87.385	99.467
P Value (Cochran's Q)	<0.001	<0.001	<0.001
Between Herd Sizes			
Cochran's Q =3.155, df=2, P=0.207			
Udder Quart -Based (n=29)			
	Small (n=10)	**Medium (n=8)**	**Large (n=11)**
Number of Udder Quarter	1765	6100	37271
Number of Udder Quarter with Subclinical Mastitis (+)	744	1901	6300
Simple Proportion	0.42	0.32	0.17
Pooled Prevalence and 95% CI	0.42 (0.33-0.51)	0.27 (0.15-0.45)	0.18 (0.12-0.25)
P Value	0.078	**0.012**	**<0.001**
Heterogeneity Test			
Cochran's Q	118.494	1074.647	2437.061
df(Cochran's Q)	9	7	10
τ^2	0.319	1.246	0.582
I^2	92.405	99.349	99.590
P Value (Cochran's Q)	**<0.001**	**<0.001**	**<0.001**
Between Herd Sizes			
Cochran's Q =15.922, df=2, P<0.001			

df= degree of freedom, C.I.: Confidence Interval, SE: Standard Error, I^2: Ratio of variance in observed effects to variance in true effects rather than sampling error, τ^2: Variance in true effect sizes.

Table 8. Multivariate meta-regression model of the prevalence of cow-based and quarter-based subclinical mastitis in Türkiye.

Cow-Based Covariate Years	Intercept	SE	95% CI		Z-Statistic	P-Value
1988-1999 (Referance)	0.00	-	-	-	-	-
2000-2009	1.22	0.69	-0.13	2.57	1.78	0.076
2010-2019	1.63	0.71	0.25	3.02	2.31	0.021
Herd Sizes						
Large (Referance)	0.00	-	-	-	-	-
Medium	0.40	0.51	-0.59	1.40	0.80	0.425
Small	0.68	0.45	-0.20	1.57	1.51	0.131
Constant	-1.81	0.63	-3.04	-0.58	-2.87	0.004
Model Test						
Q = 9.42, sd = 4, P = 0.052						
Goodness of Fit Test						
Tau² = 1.077, Tau = 1.038, I^2 = 97.97%, Q = 1326.86, df = 27, P<0.001						
Total Between-Study Variance (Constant Only)						
Tau² = 1.496, Tau = 1.223, I^2 = 98.60%, Q = 2210.22, df = 31, P<0.001						
Ratio of Explained Variance						
R^2 analog = 0.28						

Quarter-Based Covariate Region	Intercept	SE	95% CI		Z-Statistic	P-Value
The Marmara Region (Referance)	0.00	-	-	-	-	-
The Mediterranean region	0.60	0.62	-0.61	1.82	0.97	0.331
The Eastern Anatolia Region	0.69	0.49	-0.27	1.65	1.40	0.160
The Aegean Region	1.57	0.61	0.38	2.76	2.59	0.010
The Central Anatolia Region	1.73	0.64	0.48	2.98	2.72	0.007
Years						
1988-1999 (Referance)	0.00	-	-	-	-	-
2000-2009	1.08	0.50	0.11	2.05	2.18	0.029
2010-2019	1.38	0.50	0.41	2.35	2.78	0.005
Herd Size						
Large (Referance)	0.00	-	-	-	-	-
Medium	0.50	0.38	-0.25	1.25	1.31	0.191
Small	0.97	0.36	0.27	1.67	2.72	0.007
Constant	-3.40	0.64	-4.67	-2.14	-5.29	<0.001
Model Test						
Q = 33.16, df= 8, P < 0.001						
Goodness of Fit Test						
Tau² = 0.512, Tau = 0.715, I^2 = 98.83%, Q = 1711.85, df = 20, P < 0.001						
Total Between-Study Variance (Constant Only)						
Tau² = 0.731, Tau = 0.855, I^2 = 99.33%, Q = 4175.77, df = 28, P < 0.001						
Ratio of Explained Variance						
R^2 analog = 0.30						

df= degree of freedom, C.I.: Confidence Interval, SE: Standard Error, I^2: Ratio of variance in observed effects to variance in true effects rather than sampling error, Tau²: Variance in true effect sizes.

Discussion and Conclusion

The prevalence values calculated in studies conducted in Türkiye on subclinical mastitis showed a wide range between 5% and 78% in cow-based studies, and between 2% and 78% in udder quarter-based studies. With this study, a stronger and more precise estimation was provided by eliminating the inconsistencies in the individual studies regarding the effect size of the subclinical mastitis prevalence in the population. As a result of meta-analysis, the prevalence of subclinical mastitis was calculated as 48% in cow-based studies, and as 32% in udder quarter-based studies. In a meta-analysis study, the prevalence of subclinical mastitis was calculated as 41% in India (11). In a study performed by Bangar et al. (3), the prevalences of cow-based and udder quarter-based subclinical mastitis in India were calculated as 46% and 23%, respectively Getaneh and Gebramedhin (7), reported the prevalence of subclinical mastitis as 37% in Ethiopia in a meta-analysis study. In this study, the prevalence of subclinical mastitis in Türkiye was found to be similar to or even higher than in the aforementioned countries where the studies were conducted for the same purpose.

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Findings obtained in subgroup analyses based on geographical regions in Türkiye, are in line with the view of Philpot and Nickerson who conducted a study on the geographical evaluation of the prevalence of subclinical mastitis and suggested that latitude may have an effect on the prevalence of the disease. In the same study, higher tendency in somatic cell count was reported in South America where the temperature and humidity are high (18). In a meta-analysis study comparing five regions in India in terms of the prevalence of subclinical mastitis, the effect of latitude was investigated and it was reported that the Eastern (47%) and Southern (50%) regions have a higher prevalence than the Western (37%) and Northern (39%) regions (11).

In the udder quarter-based analysis, the reason for the Central Anatolia Region having the highest prevalence can be explained as administrative factors in dairy enterprises in the Central Anatolia Region and by the infection in more than one udder quarter of a cow with mastitis. In addition, since the exclusion of the Southeastern Anatolia Region and the Black Sea Region from the evaluation due to the lack of sufficient number of studies for creating a subgroup, a new subgroup analysis for region comparisons became necessary.

Bangar et al. (3) calculated the disease prevalence in local breeds (24.2%) to be lower than that of crossbreed (31.4%). This result can be interpreted as local breeds having more adaptability and more resistant than culture breeds as well as lower milk yield. Tuke et al. (19), calculated the prevalence of mastitis for local breeds (17.64%) to be lower than exotic breeds (61.51%) and explained the reason for this as certain psychological and anatomical differences between breeds. It has been determined that the highest prevalence is in Holstein cows and crossbreeds, and it has been suggested that this is due to their drooping udders, high milk yield and relatively more open teat canals (19). In contrast, Hoque et al. (10) reported in their study that this difference between breeds may be due to different environmental conditions and administrative factors. Biffa et al. (4), also reported that the prevalence of subclinical mastitis in Zebu x Holstein crossbreeds and Jersey cows was lower than the prevalence in local breeds. In this study, the prevalence of subclinical mastitis in cow-based studies in Türkiye was calculated to be higher in Holsteins and their crossbreeds compared to other breeds, which is similar to what other studies also reported. This result can be explained by the fact that local breeds have more adaptability and disease resistance than culture breeds, and that their milk yield is lower (12).

In both cow-based and the udder quarter-based studies the prevalence of subclinical mastitis tends to increase over the years. Krishnamoorthy et al. (11), showed that the prevalence of subclinical mastitis tends to increase by performing subgroup analysis compared to the years 2005-2010 and 2011-2016 (29% and 45%, respectively). The increase in the prevalence of mastitis in recent years can be explained by the extensive breeding of culture-breed cows in the world and in Türkiye, and the low disease resistance of these cows despite their high milk yield. However, Philpot and Nickerson reported in their study that the prevalence of mastitis has decreased in many countries in recent years (18).

When the prevalence of mastitis is evaluated according to herd size in Türkiye, it has been observed that large scale herds have a lower rate than medium and small-scale herds in both cow and udder quarter-based studies. This result can be explained by the fact that small-scale herds do not have the quality of management available to large-scale herds and that milk production is higher in large-scale herds (18). Additionally, this result was supported by the studies reporting that the mean somatic cell counts in the herd decrease with the increasing herd size (2, 15, 16).

R^2 analog is the ratio of the variance explained by the independent variables to the total variance in a meta-regression model. In this study, the models with the highest R^2 analog value were used in the multivariate meta-regression models created to calculate the heterogeneity between studies.

According to the multivariate meta-regression model created to determine the factors affecting the prevalence of cow-based subclinical mastitis in Türkiye, only the variables of years and herd sizes were found to be effective on the prevalence of subclinical mastitis, and it was determined that the variance observed in the created model reflected 28% of the true variance. In the udder quarter-based studies, this rate was calculated as 30% and the variables of regions, years and herd sizes were included in the model. These results showed that models created with regions, years and herd sizes are not sufficient to explain the prevalence of subclinical mastitis.

In this study, the prevalence of subclinical mastitis in dairy cows in Türkiye was evaluated by meta-analysis and the common prevalences were calculated. In this way, an opportunity was obtained to make strong and precise predictions with a large sample. It was determined that the prevalence of subclinical mastitis in Türkiye increased in the years 2010-2019 compared to the years 1988-1999 and 2000-2009. In contrast, the prevalence of subclinical mastitis in Finland has decreased from 22.3% (1991) and 20.1% (2001) to 19% (2010) in the last 20 years (9). In addition, it was clearly shown that the prevalence of subclinical mastitis in Türkiye [0.48 (95% CI: 0.41- 0.56)] is significantly higher than in India [0.41 (95% CI: 0.33-0.49)] and in Ethiopia [0.37 (95% CI: 0.33-0.41)] (7, 11).

Considering the importance of a fully integrative large farm structure in the control of diseases in dairy

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cattle breeding, examining the management mechanisms of these enterprises and modeling similar mechanisms by small enterprises will be effective in reducing subclinical mastitis. Due to the multifactorial nature of subclinical mastitis, it is important to keep a holistic approach in the combat against this disease and develop a multi-faceted control mechanism. In addition, it is necessary to conduct meta-analyses of studies examining different factors and to calculate the effects of these factors on the prevalence of subclinical mastitis.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

AA supervised, conceptualizated, administrated project. EÇG conducted formal analysis, writed original draft, investigated and visualizated. EÇG ve AA contributed to the interpretation of the results. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

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

Ethical Statement

This study does not present any ethical concerns.

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