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## Meta analysis of the prevalence of subclinical mastitis in dairy cattle

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**ABSTRACT:** Subclinical mastitis is still considered one of the most important problems affecting the dairy industry. Therefore, studies on subclinical mastitis continue to be conducted. The presence of too many studies conducted worldwide to determine the prevalence and risk factors of mastitis has necessitated systematic review or meta-analysis studies on this subject. In this study, it was aimed to perform the meta-analysis for the prevalence of cow and udder quarter-based subclinical mastitis in 193 studies conducted around the world, and to determine the effects of some factors on the prevalence of subclinical mastitis using subgroup analysis and meta-regression methods. Because high heterogeneity was detected between studies in the analysis, the random effects model (Der Simonian-Laird method) ( $Q=15149.869$ ,  $df=188$ ,  $p<0.001$ ,  $I^2=98.759$ ;  $Q=72142.706$ ;  $df=174$ ,  $p<0.001$ ;  $I^2=99.774$ ) was used in this study. The publication biases in the study samples were determined by the use of Egger's linear regression test, Begg and Mazumdar rank correlation test and funnel plots. As a result of the study, the pooled subclinical mastitis prevalence in cow and udder quarter-based studies were calculated as 0.46 (95% CI: 0.43-0.48) and 0.32 (95% CI: 0.30-0.34) respectively. The meta-analysis conducted in this study has enabled the elimination of inconsistencies in the prevalence of subclinical mastitis in individual studies conducted around the world, and has provided a stronger and more precise estimate.

**Keywords:** Meta-analysis; subclinical mastitis; dairy cow; meta-regression; prevalence

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## INTRODUCTION

Mastitis is the inflammatory reaction of the mammary gland in dairy cows to infectious agents such as bacteria, yeast, fungi and viruses, and unlike other diseases, it has been defined as a disease that can be caused by more than 140 different microorganisms (Philpot and Nickerson 2000; Baştan, 2002). In addition, it has been reported that other factors depending on the cow (breed, lactation stage, udder structure, body condition score, etc.) and the environment (shelter type, floor structure and hygiene, temperature, humidity, etc.) are also effective on the prevalence of mastitis (Kaymaz et al., 2016).

The form of mastitis, in which milk or udder abnormalities or secondary clinical signs can be observed, is defined as clinical whereas the invisible form that can be diagnosed by the detection of inflammatory cells formed by the mammary tissue using test methods is defined as subclinical mastitis. Subclinical mastitis, which is more common than clinical mastitis, has been accepted as the most important problem of dairy cattle, especially for cows with high milk yield, due to the high risk of contamination and the decrease in milk yield and quality that results in serious economic losses (Blosser, 1979; Baştan, 2013; Kaymaz et al., 2016). It has been reported that a milk yield loss of more than 10% occurs in cows with mastitis for the duration of lactation (Hortet and Seegers 1998). On the other hand, it has been reported that when the average SCC is reduced from 300.000 cells/ml to 150.000 cells/ml in a herd, milk yield increases by 238 liters per cow and provide approximately \$16,700 profit in a herd of 100 cows (Philpot and Nickerson, 2000; Baştan, 2002; Kaymaz et al., 2016).

Since subclinical mastitis has not been completely eradicated to date, interest in subclinical mastitis still continues. However, it has been reported that the versatile mastitis control programs being developed continuously with the aim of preventing subclinical mastitis in dairy farms reduce the prevalence of the disease and the economic losses caused by it (Philpot and Nickerson, 2000). Therefore, the number of studies on this subject has been increasingly continued. Östensson et al. (2013) calculated the prevalence of cow-based subclinical mastitis as 89% in dairy cows in their study conducted in Vietnam. In a study performed in Uganda by Björk (2013) the prevalence of subclinical mastitis was calculated as 90%. Searches in the Scopus database with the specific key words ('Subclinical mastitis', 'Dairy cattle' and 'Preva-

lence') showed an increase in published studies on subclinical mastitis in dairy cows (Fig. 1).

As the number of scientific studies carried out for estimating the prevalence of subclinical mastitis and determining the factors affecting the disease increased, the meta-analysis method was developed because of the need for more accurate and more reliable results with a reduced risk of bias. Meta-analysis is defined as a method of combining the results of independent studies on a particular subject, performing a statistical analysis of the research findings and reinterpreting the findings (Mosteller and Colditz, 2000). The most reliable and valid parameters with the lowest variance can be estimated related to the research topic with this method (Whitehead, 2002).

In this study, it was aimed to evaluate the prevalence of subclinical mastitis in dairy cows worldwide in the last thirty years with a meta-analysis and calculate the pooled prevalence. In addition, in this study subgroup and meta-regression analyses were performed according to geographical continents (Asia, Europe, Africa, America and Oceania), dairy cow breeds (Holstein and its crossbreed, Swiss Brown and its crossbreed, Jersey and its crossbreed, local breed and its crossbreed, other breeds and unspecified breeds) year groups (1988-1999, 2000-2009, 2010-2019) and herd sizes (small, medium and large) in order to determine the sources of heterogeneity in the prevalence of subclinical mastitis.

## MATERIAL AND METHOD

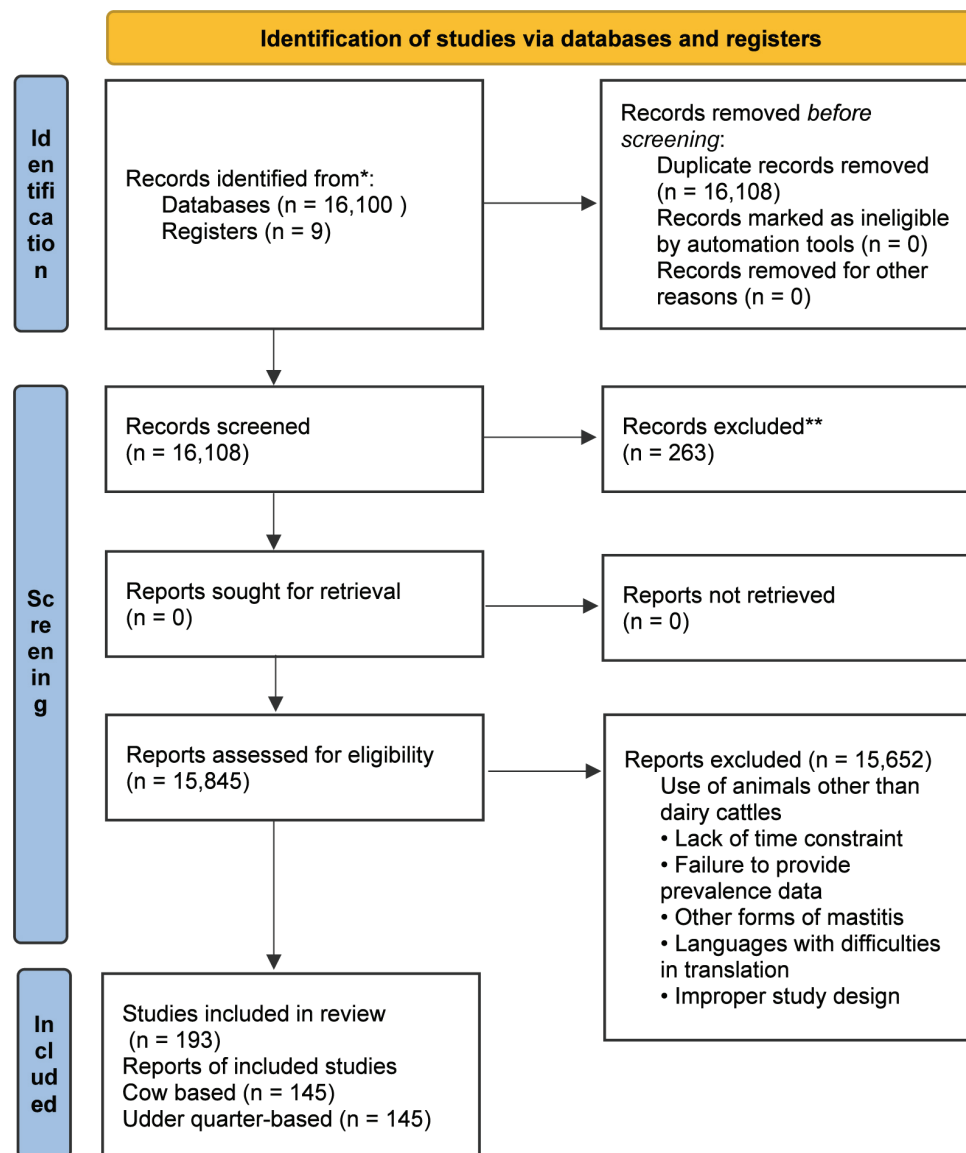
The material of the study consisted of 193 studies presenting subclinical mastitis prevalence data of dairy cows conducted around the world in the last 30 years (1988-2019). It was determined that cow and udder quarter data were both used as material to determine the prevalence of subclinical mastitis in these studies. Therefore, the prevalence of subclinical mastitis in the studies to be included in the meta-analysis was divided into the categories of cow-based and udder quarter-based. 189 cow-based and 175 udder quarter-based prevalence values from a total of 193 studies meeting the inclusion criteria were used in the analyses. 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 key words were determined as 'Subclinical mastitis', 'Dairy cattle' and 'Prevalence' and the literature scans were performed with electronic databases such as Google Scholar, PubMed, ScienceDirect and Scopus. The in-

clusion criteria for the studies to be included in the meta-analysis consisted of being published in the last 30 years, using dairy cow breeds, having prevalence of subclinical mastitis that were or can be calculated, and published in languages that have the possibility of translation (Turkish, English, Spanish, Portuguese and Indonesian). The studies that would be included in the meta-analyses were determined according to the PRISMA checklist. The flowchart is given in Fig. 1 (Page et al., 2021).

Within the scope of the study, meta-analyses were performed separately based on cows and udder quarters. A total of 180,280 cows and 1,136,041 udder quarters were included in the meta-analysis. Begg and Mazumdar rank correlation test, Egger's linear

regression test and funnel graphics were used to detect publication biases in the study samples. Duval and Tweedie's trim and fill method was used to determine the number of studies that should be added to eliminate publication biases.

Cochran's Q statistic and the  $I^2$  index were used to determine the heterogeneity in the study samples.  $I^2$  was the ratio of the real variance to the observed variance. The DerSimonian-Laird method was used to determine the heterogeneity ( $\tau^2$ ) between studies. Additionally, subgroup and meta-regression analyses were performed to determine the sources of heterogeneity in the prevalence of subclinical mastitis and to determine the variation of covariates according to their categories. The dependent variable in the gen-



**Figure. 1** Flowchart of prevalences of subclinical mastitis in dairy cows (Page et al., 2021)

erated data set was cow or udder quarter-based sub-clinical mastitis prevalence, whereas the independent variables in the formed data sets were geographical continents (Asia, Europe, Africa, America, and Oceania), the breeds of the dairy cows (Holstein and its crossbreed, Swiss Brown and its crossbreed, Jersey and its crossbreed, local breed and its crossbreed, other breeds and unspecified breeds), year groups during which the studies were published (1988-1999, 2000-2009, 2010-2019) and herd sizes [small ( $n < 100$ ), medium ( $100 \leq n < 300$ ) and large ( $300 \leq n$ )].

In the meta-regression analyses applied, the method of moments was preferred for calculating the model coefficients. In the analyses, the significance level was determined as  $p < 0.05$  in order to test the hypothesis that the prevalence was not different from 0.5 as well as to test the significance of the coefficients in the meta-regression models whereas the significance level for the significance controls of Cochran's Q heterogeneity statistics was taken as  $p < 0.10$ . The softwares CMA (Comprehensive Meta-Analysis Software v3) and R 4.0.3 (<https://cran.r-project.org/>) were used in the meta-analysis.

## RESULTS

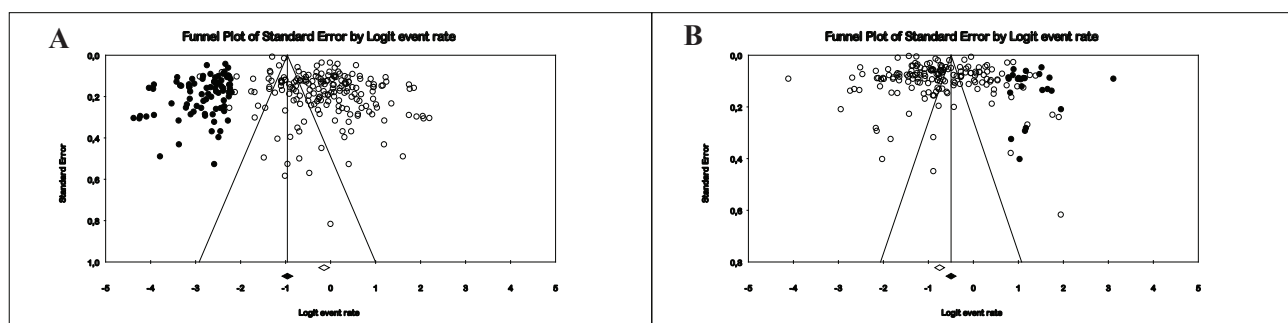
In this study, a significant heterogeneity was detected among the studies included in both the cow and udder quarter-based meta-analyses (Cochran's  $Q = 15,149.87$ ,  $df = 188$ ,  $p < 0.001$  and Cochran's  $Q = 72,142.706$ ,  $df = 174$ ,  $p < 0.001$ , respectively). The  $\tau^2$  and  $I^2$  values calculated to detect heterogeneity were calculated as 98.759 and 0.628 in cow-based and 99.774 and 0.480 in udder quarter-based studies, respectively. Due to the high heterogeneity between the included studies, the random effects model was used to calculate the size of the joint effect. The pooled prevalence of subclinical mastitis calculated based on the random effects model were 0.464 (95% CI: 0.435-0.493) in the cow-based studies and 0.321 (95% CI: 0.298-0.345) in the udder quarter-based studies (Table 1).

Funnel plots were used to detect publication bias in the study samples (Figure 2). The existence of publication bias in the study samples was shown in these funnel plots, and the missing studies were added using Duval and Tweedie's trim and fill method in order to eliminate bias. These missing studies are indicated by black dots in the graphic (Figure 2).

**Table 1.** Cow and udder quarter-based meta-analysis results of subclinical mastitis in dairy cattle worldwide

Statistics	Cow-Based	Udder Quarter-Based
Total Number	180,280	1,136,041
Number of Subclinical Mastitis (+)	51,820	278,578
Simple Ratio	0.29	0.25
Pooled Prevalence and 95% CI	0.464 (0.435-0.493)	0.321 (0.298-0.345)
P Value	0.015	<0.001
<b>Heterogeneity Test</b>		
Cochran's Q	15,149.87	72,142.706
df (Cochran's Q)	188	163
$\tau^2$	0.628	0.480
$I^2$ (%)	98.76	99.77
P Value (Cochran's Q)	<0.001	<0.001

\*df= degree of freedom, C.I.: Confidence Interval,  $I^2$ : Ratio of variance in observed effects to variance in true effects rather than sampling error,  $\tau^2$ : Variance in true effect sizes



**Figure 2.** Funnel plots of the study sample of subclinical mastitis prevalences of cow-based (A) and udder quarter-based (B)

In addition, publication bias was determined in both cow and udder quarter-based study samples according to Egger's linear regression test and Begg and Mazumdar rank correlation test ( $p < 0.001$  and  $p = 0.007$ ;  $p < 0.001$  and  $p = 0.040$ , respectively) (Table 2).

Duval and Tweedie's trim and fill method was applied because of the detection of publication bias in the study samples, and according to this method, the number of studies that should be added in order to avoid publication bias in the study samples was found as 83 for the cow-based analysis and 24 for the udder quarter-based analysis. Consequently, the corrected pooled prevalence of subclinical mastitis in cow and udder quarter based studies were calculated as 0.277 (95% CI: 0.253-0.301) and 0.379 (95% CI: 0.353-0.405), respectively (Table 3).

With the aim of determining the sources of heterogeneity, subgroup analyses were performed for the cow and the udder quarter-based studies with the variables of the 5 geographical continents on earth, breeds of dairy cow, year groups and herd sizes.

According to the results of the subgroup analysis based on geographical continents, the highest prevalence of subclinical mastitis in cow-based studies was calculated as 0.721 (95% CI: 0.585-0.826) in Oceania whereas the lowest was calculated as 0.393 (95% CI:

0.321-0.472) in Europe. However, a significant difference was found between the continents in terms of the prevalence of subclinical mastitis (Cochran's  $Q = 16.036$ ,  $df = 4$ ,  $p = 0.003$ ).

In the udder quarter-based analysis, the pooled subclinical mastitis prevalence was found to be the highest [0.467 (95% CI: 0.289-0.653)] in Oceania and the lowest [0.282 (95% CI: 0.247-0.320)] in Asia. A significant difference was found between the continents in terms of the prevalence of subclinical mastitis (Cochran's  $Q = 10.342$ ,  $df = 4$ ,  $p = 0.035$ ) (Table 4).

According to the results of the subgroup analysis based on the breeds of the dairy cows, the highest [0.509 (95% CI: 0.460-0.557)] prevalence of subclinical mastitis in the cow-based studies was calculated in the group "unspecified", which was a breed group unspecified within the scope of the study. The lowest prevalence [0.408 (95% CI: 0.337-0.483)] was calculated in the local breeds and in their crossbreeds. However, it was determined that the prevalence of subclinical mastitis did not differ significantly according to breeds (Cochran's  $Q = 5.479$ ,  $df = 5$ ,  $p = 0.360$ ). In the subgroup analysis performed according to breeds, in udder quarter-based subclinical mastitis studies, the highest pooled subclinical mastitis prevalence was found as 0.523 (95% CI: 0.246-0.785) in Jerseys and their crossbreeds and the lowest was found as

**Table 2.** Publication bias tests of cow and udder quarter-based subclinical prevalence study samples

	Egger's Linear Regression Test	Begg and Mazumdar Rank Correlation Test
<b>Cow-Based</b>		
Coefficient	6.604	-0.131
t statistics	10.129	-
zstatistics	-	2.679
p Value	<0.001	0.007
<b>Udder Quarter-Based</b>		
Coefficient	6.275	-0.108
t statistics	3.383	-
zstatistics	-	2.053
p Value	<0.001	0.040

**Table 3.** Results of Duval and Tweedie's Trim and Fill methods on cow-based and udder quarter-based subclinical mastitis prevalence study samples

	Number of Trimmed Study	Prevalence and 95% CI	Cochran's Q
<b>Cow Based</b>			
Observed Values	-	0.464 (0.435-0.493)	15,149.869
Adjusted Values	83	0.277 (0.253-0.301)	28,288.023
<b>Udder Quarter Based</b>			
Observed Values	-	0.321 (0.298-0.345)	72,142.706
Adjusted Values	24	0.379 (0.353-0.405)	88,262.420

\*Cochran's Q: The amount of heterogeneity after filling the missing studies according to the Trim and Fill method

**Table 4.** Results of subgroup analysis of subclinical mastitis prevalences based on cow and udder quarter worldwide by continents

	Continents (n=189)				
	Asia	Europe	Africa	America	Oceania
<b>Cow Based</b>					
Number of Study	98	8	61	18	4
Number of Cows in the Studies	23,533	129,576	13,180	11,513	2,478
Number of Subclinical Mastitis (+)	8,724	30,151	5,733	5,551	1,661
Simple Rate (prevalence)	0.371	0.233	0.435	0.482	0.670
Pooled Prevalence and 95% CI	0.461 (0.416-0.506)	0.393 (0.321-0.472)	0.455 (0.408-0.503)	0.481 (0.417-0.546)	0.721 (0.585-0.826)
p Value	0.090	<b>0.008</b>	0.064	0.574	<b>0.002</b>
<b>Heterogeneity Test</b>					
Cochran's Q	3,501.871	1,880.914	1,537.345	688.098	69.428
df (Cochran's Q)	97	7	60	17	3
$\tau^2$ Value	0.787	0.204	0.540	0.295	0.351
I <sup>2</sup> Value	97.230	99.628	96.097	97.529	95.679
p Value (Cochran's Q)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Between Groups</b>					
Cochran's Q =16,036, df=4, p=0.003					
<b>Continents (n=165)</b>					
<b>Udder Quarter Based</b>					
Number of Study	72	11	50	27	5
Number of Udder Quarter in the Studies	86,229	748,222	139,420	159,123	3,047
Number of Udder Quarter Subclinical Mastitis (+)	20,277	152,397	59,849	44,870	1,185
Simple Rate (prevalence)	0.235	0.204	0.429	0.282	0.389
Pooled Prevalence and 95% CI	0.282 (0.247-0.320)	0.350 (0.306-0.398)	0.329 (0.290-0.371)	0.377 (0.303-0.456)	0.467 (0.289-0.653)
p Value	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.003</b>	0.731
<b>Heterogeneity Test</b>					
Cochran's Q	7,809.282	5,702.848	6,912.430	17,363.064	291.780
df (Cochran's Q)	71	10	49	26	4
$\tau^2$ Value	0.589	0.107	0.420	0.749	0.744
I <sup>2</sup> Value	99.091	99.825	99.291	99.850	98.629
p Value (Cochran's Q)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Between Groups</b>					
Cochran's Q = 10,342, df=4, p= 0.035					

\*df= degree of freedom, CI: Confidence Interval, I<sup>2</sup>: Ratio of variance in observed effects to variance in true effects rather than sampling error,  $\tau^2$ : Variance in true effect sizes

0.242 (95% CI: 0.197-0.293) in local breeds and their crossbreeds. It was determined that the prevalence of the disease differed significantly regarding the breeds (Cochran's Q=11,848, df=5, p=0.037) (Table 5).

In cow-based subclinical mastitis studies, the highest prevalence of subclinical mastitis was observed in the 2010-2019 subgroup [0.486 (95% CI: 0.454-0.519)] whereas the lowest disease prevalence was calculated in the 1988-1999 subgroup [0.260 (95% CI: 0.088-0.560)] in the subgroup analysis by years.

It was determined that the prevalence of subclinical mastitis showed a significant difference regarding the year groups (Cochran's Q=6,507, df=2, p=0.039).

According to the subgroup analysis of udder quarter-based subclinical mastitis prevalence by year groups, the pooled subclinical mastitis prevalence was the highest in the 2010-2019 subgroup [0.336 (95% CI: 0.308-0.365)] and the lowest in the 2000-2009 subgroup (0.278 95% CI: 0.240-0.320). It was determined that the prevalence of subclinical masti-

**Table 5.** Sub group analyzes of subclinical mastitis prevalences based on cow and udder quarters worldwide by breed

	Breeds (n=189)					
	Holstein and Crossbred	Swiss Brown and Crossbred	Jersey and Crossbred	Indigenous Breed and Crossbred	Other Breed	Unspecified Breeds
<b>Cow-Based</b>						
Number of Studies	45	6	12	44	41	41
Number of Cows in the Studies	35,114	1,111	767	11,807	111,438	20,043
Number of Subclinical Mastitis (+)	11,370	475	348	3,699	26,151	9,777
Simple Rate (prevalence)	0.324	0.428	0.454	0.313	0.235	0.488
Pooled Prevalence and 95% CI	0.466 (0.415-0.518)	0.476 (0.281-0.678)	0.433 (0.319-0.554)	0.408 (0.337-0.483)	0.480 (0.410-0.550)	0.509 (0.460-0.557)
p Value	0.203	0.821	0.276	<b>0.016</b>	0.574	0.729
<b>Heterogeneity Test</b>						
Cochran's Q	2,142.912	171.267	97.122	1,942.226	3,314.288	1,657.460
df (Cochran's Q)	44	5	11	43	40	40
$\tau^2$ Value	0.462	1.058	0.609	0.997	0.811	0.386
I <sup>2</sup> Value	97.947	97.081	88.674	97.786	98.793	97.587
p Value (Cochran's Q)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Between Group</b>						
Cochran's Q=5,479, df=5, p=0,360						
<b>Breeds (n=165)</b>						
<b>Udder Quarter Based</b>						
Number of Studies	36	4	3	28	47	47
Number of Udder Quarter in the Studies	239,812	2,929	448	37,551	657,672	197,629
Number of Udder Quarter Subclinical Mastitis (+)	57,448	895	185	9,841	133,497	76,712
Simple Rate (prevalence)	0.240	0.306	0.413	0.262	0.203	0.388
Pooled Prevalence and 95% CI	0.353 (0.289-0.422)	0.338 (0.259-0.426)	0.523 (0.246-0.785)	0.242 (0.197-0.293)	0.334 (0.2890.382)	0.325 (0.287-0.365)
p Value	<0.001	<0.001	0.884	<0.001	<0.001	<0.001
<b>Heterogeneity Test</b>						
Cochran's Q	9,998.600	55.256	15.52	2,566.969	18,451.009	12,032.375
df (Cochran's Q)	35	3	2	27	46	46
$\tau^2$ Value	0.795	0.137	0.962	0.481	0.533	0.384
I <sup>2</sup> Value	99.650	94.571	87.303	98.986	99.751	99.618
p Value (Cochran's Q)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<b>Between Group</b>						
Cochran's Q=11,848, df=5, p=0.037						

\*df= degree of freedom, CI: Confidence Interval, I<sup>2</sup>: Ratio of variance in observed effects to variance in true effects rather than sampling error,  $\tau^2$ : Variance in true effect sizes

tis showed a significant difference regarding the year groups (Cochran's Q=5,097, df=2, p=0.078) (Table 6). In the subgroup analyses of cow-based subclinical mastitis studies by herd size, the highest prevalence [0.498 (95% CI: 0.451-0.544)] was observed in

small herds and the lowest prevalence [0.395 (95% CI: 0.350-0.443)] was observed in large herds. In addition, a significant difference was found between herd sizes in terms of the prevalence of the disease (Cochran's Q=11,752, df=2, p=0.003).

**Table 6.** Subgroup analysis of subclinical mastitis prevalences based on cow and udder quarter by years

	Years (n=189)		
	1988-1999	2000-2009	2010-2019
<b>Cow-Based</b>			
Number of Studies	5	39	145
Number of Cows in the Studies	6,261	17,260	156,759
Number of Subclinical Mastitis (+)	1,847	6,732	43,241
Simple Rate (prevalence)	0.295	0.390	0.276
Pooled Prevalence and 95% CI	0.260 (0.088-0.560)	0.410 (0.351-0.473)	0.486 (0.454-0.519)
p Value	0.111	<b>0.005</b>	0.410
<b>Heterogeneity Test</b>			
Cochran's Q	1,325.739	2,087.690	11,192.458
df (Cochran's Q)	4	38	144
$\tau^2$ Value	2.134	0.609	0.580
I <sup>2</sup> Value	99.684	98.060	98.635
p Value (Cochran's Q)	<0.001	<0.001	<0.001
<b>Between Groups</b>			
Cochran's Q =6,507, df=2, p=0,039			
	Years (n=165)		
<b>Udder Quarter Based</b>			
Number of Studies	10	40	115
Number of Udder Quarter in the Studies	58,088	69,573	1,008,380
Number of Udder Quarter Subclinical Mastitis (+)	18,913	20,793	238,872
Simple Rate (prevalence)	0.326	0.299	0.237
Pooled Prevalence and 95% CI	0.321 (0.214-0.451)	0.278 (0.240-0.320)	0.336 (0.308-0.365)
p Value	0.008	<0.001	<0.001
<b>Heterogeneity Test</b>			
Cochran's Q	5,850.388	4,891.370	56,693.328
df (Cochran's Q)	9	39	114
$\tau^2$ Value	0.788	0.405	0.472
I <sup>2</sup> Value	99.846	99.203	99.799
p Value (Cochran's Q)	<0.001	<0.001	<0.001
<b>Between Group</b>			
Cochran's Q=5,097, df=2, p=0.078			

\*df= degree of freedom, CI: Confidence Interval, I<sup>2</sup>: Ratio of variance in observed effects to variance in true effects rather than sampling error,  $\tau^2$ : Variance in true effect sizes

In the subgroup analyses of udder quarter-based subclinical mastitis studies regarding herd size, the highest prevalence [0.408 (95% CI: 0.339-0.482)] was found in the small herd subgroup and the lowest prevalence [0.266 (95% CI: 0.234-0.301)] was found in the large herd subgroup. A significant difference was found between herd sizes in terms of the prevalence of the disease (Cochran's Q=14,355, df=2, p=0.001) (Table 7).

In the study, multivariate meta-regression models were formulated for both the studies based on cows and the studies based on udder quarters. Among the multivariate meta-regression models formulated for cow-based subclinical mastitis studies, the multivariate model with the highest R<sup>2</sup> analog statistics was obtained using the variables of geographic continents and herd sizes. The generated model explains 39% of the true variance (R<sup>2</sup> analog=0.39). According to this model, the prevalence of the disease in the Oceania

**Table 7.** Subgroup analysis of cow and udder quarter-based subclinical mastitis prevalences by herd size

	<b>Herd Size (n=189)</b>		
	<b>Small</b>	<b>Medium</b>	<b>Large</b>
<b>Cow-Based</b>			
<b>Number of Studies</b>	57	77	55
<b>Number of Cows in the Studies</b>	3,368	14,760	162,152
<b>Number of Subclinical Mastitis (+)</b>	1,682	7,095	43,043
<b>Simple Rate (prevalence)</b>	0,499	0,481	0,265
<b>Pooled Prevalence and 95% CI</b>	0.498 (0.451-0.544)	0.493 (0.451-0.535)	0.395 (0.350-0.443)
<b>p Value</b>	0.919	0.746	<b>&lt;0.001</b>
<b>Heterogeneity Test</b>			
<b>Cochran's Q</b>	352.936	1,756.941	10,094.019
<b>df (Cochran's Q)</b>	56	76	54
<b><math>\tau^2</math> Value</b>	0.409	0.538	0.537
<b>I<sup>2</sup> Value</b>	84.133	95.674	99.465
<b>p Value (Cochran's Q)</b>	<0.001	<0.001	<0.001
<b>Between Groups</b> Cochran's Q=11,752, df=2, p=0,003			
<b>Herd Size (n=165)</b>			
<b>Udder Quarter Based</b>			
<b>Number of Studies</b>	42	64	59
<b>Number of Udder Quarter in the Studies</b>	11,640	47,298	1,077,103
<b>Number of Udder Quarter Subclinical Mastitis (+)</b>	4,035	15,910	258,633
<b>Simple Rate (prevalence)</b>	0.347	0.336	0.240
<b>Pooled Prevalence and 95% CI</b>	0.408 (0.339-0.482)	0.324 (0.284-0.367)	0.266 (0.234-0.301)
<b>p Value</b>	0.015	<0.001	<0.001
<b>Heterogeneity Test</b>			
<b>Cochran's Q</b>	1,914.550	5,316.906	61,844.917
<b>df (Cochran's Q)</b>	41	63	58
<b><math>\tau^2</math> Value</b>	0.933	0.616	0.316
<b>I<sup>2</sup> Value</b>	97.859	0.591	99.906
<b>p Value (Cochran's Q)</b>	<0.001	<0.001	<0.001
<b>Between Group</b> Cochran's Q =14,355, df=2, p=0.001			

\*df= degree of freedom, CI: Confidence Interval, I<sup>2</sup>: Ratio of variance in observed effects to variance in true effects rather than sampling error,  $\tau^2$ : Variance in true effect sizes

continent was 1.25-fold higher than in the European continent ( $p<0.001$ ). The prevalence of subclinical mastitis in small and medium sized herds were 0.47 and 0.43 folds, respectively, higher than the prevalence in large herds ( $p<0.001$ ) (Table 8).

Among the multivariate meta-regression models formulated for udder quarter-based subclinical mastitis studies, the multivariate model with the highest R<sup>2</sup> analog statistics was obtained using the variables

of geographic continents and herd sizes. The generated model explains 25% of the true variance (R<sup>2</sup> analog=0.25). According to this model, the prevalence of the disease in the Africa continent was 0.17-fold lower than the prevalence of the disease in the Europe continent, and the prevalence in the Asian continent was 0.46-fold lower than the prevalence in the European continent ( $p=0.414$ ,  $p=0.021$ , respectively). Compared to the European continent, the prevalence of subclinical mastitis in America was 0.10-fold

higher, and the prevalence in the continent Oceania was 0.19-fold higher ( $p=0.636$   $p<0.569$ , respectively). The subclinical mastitis prevalence in small and medium sized herds were 0.69-fold and 0.37-fold

higher, respectively, than that of the large sized herds ( $p<0.001$ ). According to the model test, the prevalence of subclinical mastitis differed significantly between the subgroups ( $Q=51.05$ ,  $df=6$ ,  $p<0.001$ ) (Table 8).

**Table 8.** Multivariate meta-regression model of cow-based and udder quarter-based subclinical mastitis prevalences worldwide

Covariate	Coefficient	St Error	95% CI		z-Statistics	p-Value
<b>Continents</b>						
Europe (Reference)	0.00	-	-	-	-	-
Africa	0.01	0.11	-0.20	0.23	0.13	0.898
America	0.19	0.17	-0.13	0.52	1.16	0.246
Asia	0.04	0.24	-0.44	0.51	0.16	0.876
Oceania	1.25	0.33	0.60	1.89	3.76	<0.001
Q=15.23, df=4, p=0.004						
<b>Herd Size</b>						
Large (Reference)	0.00	-	-	-	-	-
Small	0.47	0.13	0.21	0.73	3.51	<0.001
Medium	0.43	0.12	0.21	0.66	3.74	<0.001
Constant	-0.51	0.11	-0.72	-0.30	-4.76	<0.001
Q=17.23, df=2, p<0.001						
<b>Test of Model</b>						
Q = 30.77, df = 6, p<0.001						
<b>Model Goodness of Fit Test</b>						
Tau <sup>2</sup> = 0.384, Tau = 0.620, I <sup>2</sup> = 97.51%, Q = 7,321.80, df = 182, p<0.001						
<b>Total Between-Study Variance (Constant Only)</b>						
Tau <sup>2</sup> = 0.628, Tau = 0.792, I <sup>2</sup> = 98.76%, Q = 15,149.87, df = 188, p<0.001						
<b>Ratio of Explained Variance</b>						
R <sup>2</sup> analog = 0.39						
<b>Udder Quarter Based</b>						
<b>Continents</b>						
Europe(Reference)	0.00	-	-	-	-	-
Africa	-0.17	0.21	-0.57	0.24	-0.82	<b>0.414</b>
America	0.10	0.22	-0.32	0.53	0.47	<b>0.636</b>
Asia	-0.46	0.20	-0.84	-0.07	-2.30	<b>0.021</b>
Oceania	0.19	0.33	-0.46	0.84	0.57	<b>0.569</b>
Q=23.30, df=4, p=0,001						
<b>Herd Size</b>						
Large (Reference)	0.00	-	-	-	-	-
Small	0.69	0.12	0.44	0.93	5.49	<0.001
Medium	0.37	0.11	0.16	0.58	3.41	<0.001
Constant	-0.82	0.19	-1.19	-0.45	-4.40	<0.001
Q=31.40, df=2, p<0,001						
<b>Test of Model</b>						
Q = 48.07, df = 6, p < 0.001						
<b>Model Goodness of Fit Test</b>						
Tau <sup>2</sup> = 0.356, Tau = 0.597, I <sup>2</sup> = 99.57%, Q = 39,236.45, df = 168, p<0.001						
<b>Total Between-Study Variance (Constant Only)</b>						
Tau <sup>2</sup> = 0.475, Tau = 0.689, I <sup>2</sup> = 99.77%, Q = 74,207.47, df = 174, p<0.001						
<b>Ratio of Explained Variance</b>						
R <sup>2</sup> analog = 0.25						

\*df= degree of freedom, CI: Confidence Interval, St Error: Standart Error, I<sup>2</sup>: Ratio of variance in observed effects to variance in true effects rather than sampling error,  $\tau^2$ : Variance in true effect sizes

## DISCUSSION

Within the scope of this study, the examination of the prevalence values calculated in the studies conducted on subclinical mastitis worldwide showed a heterogeneous distribution ranging from 5% to 90% in cow-based studies and ranging from 2% to 88% in udder quarter-based studies.

According to the meta-analysis results, the prevalence of cow-based and udder quarter-based subclinical mastitis worldwide was calculated as 46.4% and 32.1%, respectively. However, as a result of Duval and Tweedie's trim and fill method applied in order to eliminate publication bias in the study samples by adding the missing studies to the analysis, the prevalence were corrected as 27.7% and 37.9% in cow-based and udder quarter-based studies respectively.

Bangar et al. (2014) in their meta-analysis study performed on the studies conducted in the Punjab, Haryana, Uttar Pradesh, Madhya Pradesh and Maharashtra regions in India, calculated the cow-based subclinical mastitis prevalence as 46.35% and udder-based prevalence as 23.25%. In a meta-analysis conducted by Krishnamoorthy et al. (2017), the prevalence of subclinical mastitis was calculated as 41% in India. Getaneh and Gebremedhin (2017) reported the prevalence of subclinical mastitis in Ethiopia as 37% in their meta-analysis study. In this study, the worldwide pooled subclinical mastitis prevalence was calculated as similar to or even higher than the prevalence calculated in the previous studies conducted on a national basis for the same purpose.

Krishnamoorthy et al. (2021), estimated the pooled subclinical mastitis prevalence as 42% (95% CI: 39 - 45), the Cochrane's Q value as 23489.9 and  $I^2 = 99.4$  and  $\tau^2 = 0.912$  ( $P < 0.01$ ) in their meta-analysis study conducted with 206 subclinical mastitis studies published worldwide between 1967 and 2019. In our study, Cochrane's Q value was calculated as 15149.87,  $I^2 = 98.759$  and  $\tau^2 = 0.628$  ( $P < 0.001$ ). It was observed that the heterogeneity among the studies included in our study was higher.

According to the subgroup analyses regarding the geographical continents around the world, the lowest pooled subclinical mastitis prevalence value in cow-based studies was calculated in Europe (39%) whereas the highest in the Oceania continent (72%). Krishnamoorthy et al. (2021), in their subgroup analysis according to continents in a cow-based study, calculated the highest prevalence in the North America

(46%) and the lowest prevalence in the South America (34%). In the present study, the highest prevalence in udder quarter-based studies was calculated in the continent of Oceania, the lowest prevalence was calculated as 28% in the continent of Asia, and the pooled prevalence in the continent of Europe was calculated as 35%. The high prevalence in Oceania may be due to the low number of studies conducted there, the high prevalence of subclinical mastitis observed in these studies and the inclusion of Indonesia in Oceania. In some of the studies, the prevalence of subclinical mastitis in Indonesia was calculated as 85.33% (Effendi et al. 2018) and as 58.18% (Zalizar et al., 2018). Krishnamoorthy et al. (2021) calculated the prevalence of subclinical mastitis in the continents of Asia, Europe and Oceania as 42%, 37% and 36%, respectively.

The pooled prevalence calculated for the Asian continent (28%) in this study was lower than the findings of the study carried out by the Krishnamoorthy et al. (2017) who compared five regions of India [East (47%), South (50%), West (37%) and North (39%)] in terms of subclinical mastitis prevalence. In this study, the prevalence of subclinical mastitis in the African continent based on 61 cow-based studies were calculated as 46%. Getaneh and Gebremedhin (2017) conducted a meta-analysis of 39 studies in Ethiopia and calculated the prevalence of subclinical mastitis as 37%. The latitude may be effective on the prevalence calculated in the geographical evaluation of subclinical mastitis prevalence supports the results obtained in the study of Philpot and Nickerson (2000) who indicated that somatic cell count has a tendency to be higher in South America, where the temperature and humidity are higher than those of the traditional dairy regions.

In the subgroup analysis performed according to breeds, the lowest prevalence of subclinical mastitis was calculated in local breeds and their crossbreeds (41%), and the highest prevalence among the specified breeds was calculated in the Swiss Brown breed and its crossbreeds (47.6%). Around the world, the lowest prevalence of subclinical mastitis was found in local breeds and mixed breeds (41%) and the highest prevalence among certain breeds was determined in Swiss Brown and their crossbreeds (47.6%) on a cow basis in the subgroup analysis based on breeds. This result was interpreted as the fact that indigenous breeds have more adaptability and disease resistance along with lower milk yield compared to dairy cow breeds

(Lakew et al., 2009). Tuke et al. (2017) calculated the mastitis prevalence of the indigenous breed (17.64%) to be lower than that of the exotic breed (61.51%), and explained the reason behind this as certain physiological and anatomical differences between the breeds. In addition, Tuke et al. (2017) evaluated the position of the teats and udder and the anatomy of the teat canal as the reason why exotic breeds are more susceptible to mastitis. In another study, it was suggested that this difference between the breeds may be due to other uncontrollable factors such as the cows being in different conditions and the managements of the dairy enterprises (Lakew et al., 2009). Bangar et al. (2014), in their meta-analysis study, calculated the prevalence of subclinical mastitis in local breeds (24.2%) to be lower than that of mixed breeds (31.4%). However, Biffa et al. (2005) reported in their study that the prevalence of subclinical mastitis in Zebu x Holstein crossbreed and Jersey cows were lower than the prevalence of the disease in local breeds.

In the subgroup analyses performed according to breeds on udder quarter-based studies worldwide, the highest prevalence was calculated in Jersey cows and their crossbreeds (52%) and the lowest prevalence was calculated in local breeds and their crossbreeds (24%). Hoque et al. (2014) found the highest prevalence in their study in Holstein cows and their crossbreeds, and the lowest prevalence in the local Zebu breed, and they reported that this high prevalence was due to the pendulous udders, high milk yield and relatively open teat canals of Holstein cows and their crossbreeds.

The prevalence of subclinical mastitis in cow-based studies worldwide has shown an increasing tendency, and reached the highest level in recent years. The lowest prevalence value was calculated between 1988 and 1999 (26%) and the highest prevalence value was calculated between 2010 and 2019 (49%). Similarly, Krishnamoorthy et al. (2017) in a meta-analysis study covering the years 2005-2016, reported that the prevalence of subclinical mastitis in India raised from 29% to 45% in the 2011-2016 period, and that there was an increase in the prevalence of subclinical mastitis over the years. In another study, the subclinical mastitis prevalence for the year groups 1967-2000, 2001-2010 and 2011-2019 were estimated as 40%, 38% and 43% respectively, and it was shown that there was an increase in the prevalence of the disease in recent years (Krishnamoorthy et al., 2021).

According to the udder quarter-based studies, the

lowest prevalence value was calculated in 2000-2009 (27.8%) and the highest prevalence value was found in 2010-2019 (33.6%). Around the world, a 20.9% increase was observed in the prevalence of udder quarter-based disease from the first 10-year period to the last 10-year period. This increase in the prevalence of mastitis in recent years may be due to high-yielding dairy cows becoming more widespread around the world and their lower resistance to diseases.

In the subgroup analyses of subclinical mastitis prevalence based on the size of the herd, in both cow-based and udder quarter-based studies, the highest prevalence was calculated in small herds (49.8%, 40.8%, respectively) and the lowest prevalence was calculated in large herds (39.5% and 26.6%, respectively). Philpot and Nickerson (2000) attributed this situation to the fact that small herds do not have the high quality of management practices that are available in large herds as well as high milk production in large herds. In some studies, it has been reported that the average somatic cell count in the herd decreased with the increasing herd size (Allore et al., 1997; Oleggini et al., 2001). This showed clearly that large-scale enterprises implement mastitis control programs better. In reducing the prevalence of subclinical mastitis, the herd management mechanisms of large-scale enterprises becoming models for small enterprises and supporting the small enterprises for good management practices is of importance.

In this study, continents and herd size factors were found significant according to a meta-regression model formulated for factors that influence the prevalence of cow-based subclinical mastitis worldwide. According to the  $R^2$  analog values of the model generated with these factors, it has been determined that the observed variance reflects 39% of the variance between the true effect sizes. This rate was calculated to be lower (25%) in udder quarter-based studies. The  $R^2$  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 values were used in the multivariate meta-regression models formulated to calculate the heterogeneity between studies. However, the low  $R^2$  analog values calculated in this study showed that the factors constituting the subgroups were insufficient to explain the heterogeneity between studies.

In this study, the prevalence of subclinical mastitis in the world was analyzed in the most general framework and the results were discussed among

themselves due to the low number of studies conducted with the same purpose. With the meta-analysis performed in the study, the inconsistencies in the prevalence of subclinical mastitis in individual studies conducted around the world were eliminated and a stronger and more precise prediction was provided. However, the need for meta-analysis studies to be performed with studies that deal with more factors effective in combating subclinical mastitis in dairy cattle has also been emerged. It has also emerged that meta-analysis of studies that deal with more factors effective in combating subclinical mastitis in dairy cattle should be done.

The multifactorial nature of subclinical mastitis requires the examination of variables other than the ones discussed in this study to be able to control the disease. In order to obtain more comprehensive and accurate results, it is necessary to include more factors in the meta-analysis of primary studies conducted to evaluate the prevalence of subclinical mastitis.

However, these variables were not included in the meta-analysis due to the insufficiency of studies that included all variables such as ages of cows, lactation

periods, body condition scores, udder structures, variable types, floor structure and hygiene, and environmental temperature. Moreover the differences in the categorization of some numerical variables caused the insufficient amount of data to form the subgroups. Therefore the inability to include these variables in the analysis was considered as a limitation of the study.

## CONCLUSIONS

In this study, some factors affecting the prevalence of subclinical mastitis were determined. However, subclinical mastitis has a multifactorial nature. Therefore, further meta-analysis studies are needed that examine other factors and determine the effects of these factors on the prevalence of subclinical mastitis.

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## CONFLICT OF INTEREST

The authors declare no competing interests.

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