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Metaanalysis of subclinical mastitis prevalence in water buffaloes (*Bubalus bubalis*)

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Abstract: Water buffalo milk, compared to cow's milk, has a preferable composition in terms of health and nutritional value. Subclinical mastitis, one of the most important problems of the dairy industry, is as important of an issue in water buffaloes as in cows. In this study, it was aimed to evaluate the prevalence of subclinical mastitis in buffaloes by metaanalysis, and to calculate the pooled prevalence. For the metaanalysis of the prevalence of subclinical mastitis in buffaloes, a total of 382 publications 294 from Scopus, 31 from Medline Complete, and 57 from ScienceDirect were obtained as a result of the literature searches with the key words "Buffalo, Water buffalo, Nili Ravi, *Bubalus bubalis*, Asian buffalo, Jafarabadi, Italian Mediterranean buffalo, subclinical mastitis prevalence". Five repeating studies were excluded. The study material is made up of 53 subclinical mastitis prevalence data derived from the 51 studies in total that were conducted between 1989 and 2020. In this study, 10,996 water buffaloes (water buffalo-based studies) and 44,372 udder quarters (udder quarter-based studies) were included in the metaanalysis. Additionally, a total of 3292 agent identification results from 38 prevalence data obtained from 14 studies were used for metaanalysis of subclinical mastitis prevalence by isolates. A high level of heterogeneity ($I^2(\%) > 75\%$) was detected amongst both the water buffalo-based studies and the udder quarter-based studies that were included in the analyses. At the end of the study, subclinical mastitis pooled prevalence was calculated to be 0.38 (95% CI: 0.32–0.44) in water buffalo-based studies and 0.28 (95% CI: 0.23–0.34) in udder quarter-based studies. In 38 studies with agent identification, the pooled prevalence according to isolates for *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., coagulase-negative staphylococci (CNS), and *Escherichia coli* (*E. coli*) were respectively calculated as 0.36; 0.26; 0.03; 0.39, and 0.11. With this study, the inconsistencies in individual studies regarding the magnitude of the effect of the prevalence of subclinical mastitis in water buffaloes in the population were eliminated by metaanalysis, enabling us to make a stronger and more precise estimation.

Key words: Metaanalysis, prevalence, subclinical mastitis, water buffalo

1. Introduction

Water buffaloes, with their high adaptability, represent an important resource for bovine farming during climate changes and in hotter and more humid conditions. For this reason, it is believed that water buffaloes play an important role in global animal farming in order to provide high quality animal proteins for the rising human population [1].

Water buffalo farming in the world most commonly exists in the Asian continent and is mostly characterized by a traditional production system. However, European countries have adopted more modern approaches in water buffalo farming and have provided important improvements and developments; most notably with water buffalo breeding studies, but also in the fields of feeding, herd management, and health preservation [2].

Although they are land animals, water buffaloes prefer cooler environmental conditions and therefore enjoy living in wet grasslands, mud and swamps, and tropical and subtropical forests. For this reason, despite being spread throughout South Asia to Europe, they are mostly concentrated in India, Indonesia, and South Asia where the climate is most suited. The water buffalo population in the world has continuously grown in the last 20 years and has reached approximately 231.681 million in number, most of which reside in developing countries [3]. However, due to important problems such as low milk yield, high infertility rates and low calf survival rates, water buffalo farming has remained unpopular compared to cattle globally [4].

Water buffaloes are receptive to most diseases and parasites that affect cattle, but to a lesser extent [5]. Water buffaloes being the choice of animal in wet and muddy areas, being kept in unhygienic conditions, improper milking procedures, keeping healthy and unhealthy animals together and trauma inflicted on teats by

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unweaned calves all lay the groundwork for subclinical mastitis [6].

Studies regarding subclinical mastitis, one of the most important diseases affecting milk yield in water buffalo farming, have increased in recent years reaching the number 1555 in 2020 according to the Scopus database (Figure 1). These studies were mostly focused on the health and reproductive qualities of water buffaloes [7].

It was found that the prevalence values calculated in the studies done on subclinical mastitis in water buffaloes showed a lot of variation. The materials of these studies calculating prevalence sometimes consisted of water buffaloes and sometimes consisted of udder quarters. Prevalence values in water buffalo-based studies were reported to be 0.1 at minimum and 0.8 at maximum [8, 9]. In mammary lobe-based studies, they were reported to be 0.004 at minimum and 0.6 at maximum [10, 11]. It was thought that the reason for this variation could be attributed to the sample sizes. One of the methods used to appropriately combine the findings of studies conducted with small sample sizes in order to create a larger sample and be able make stronger and more precise parameter estimations is metaanalysis [12].

The purpose of this study was to evaluate the studies on the prevalence of subclinical mastitis in water buffaloes (*Bubalus bubalis*) throughout the world by metaanalysis, to calculate the pooled prevalence and to pinpoint the effects of herd population and the year in which the study was conducted using metaregression analysis. In addition, it was aimed to determine the pooled prevalence according to the isolates obtained from subclinical mastitis studies in which the agent was identified in the second stage of the study.

2. Materials and methods

For the metaanalysis of the prevalence of subclinical mastitis in water buffaloes, there were a total of 382 publications found, 294 of which were found on Scopus, 31 on Medline Complete, and 57 on ScienceDirect, using the key words "Buffalo, Water buffalo, Nili Ravi, *Bubalus bubalis*, Asian buffalo, Jafarabadi, Italian Mediterranean buffalo, Subclinical mastitis prevalence" as search terms. Five duplicate studies were excluded with the help of Covidence (www.covidence.org/) the systematic review manager. The inclusion criteria for metaanalysis were set as "Using water buffaloes (*Bubalus Bubalis*) as animal material, subclinical mastitis prevalence calculated or can be calculated".

Some of the studies conducted in order to determine the prevalence of subclinical mastitis in water buffaloes use udder quarters as study material and not water buffaloes, and vice versa. Because of this, in this study, metaanalysis has been separately applied to water buffalo-based studies and udder quarter-based studies. As a result of the literature review, a total of 10,996 water buffaloes from the 34 water buffalo-based studies that met the inclusion criteria and a total of 44,372 udder quarters from the 33 udder quarter-based studies were deemed fit (Tables 1 and 2, respectively) along with the 3292 isolates from the 14 studies (Table 3) with agent identification were included in the metaanalysis.

In the metaanalysis, the effect size was taken as the prevalence of subclinical mastitis, and a random effect model was used since high heterogeneity was detected between studies. The Cochran Q statistic and I^2 index was used to determine the heterogeneity amongst the studies; the DerSimonian-Laird method was used to

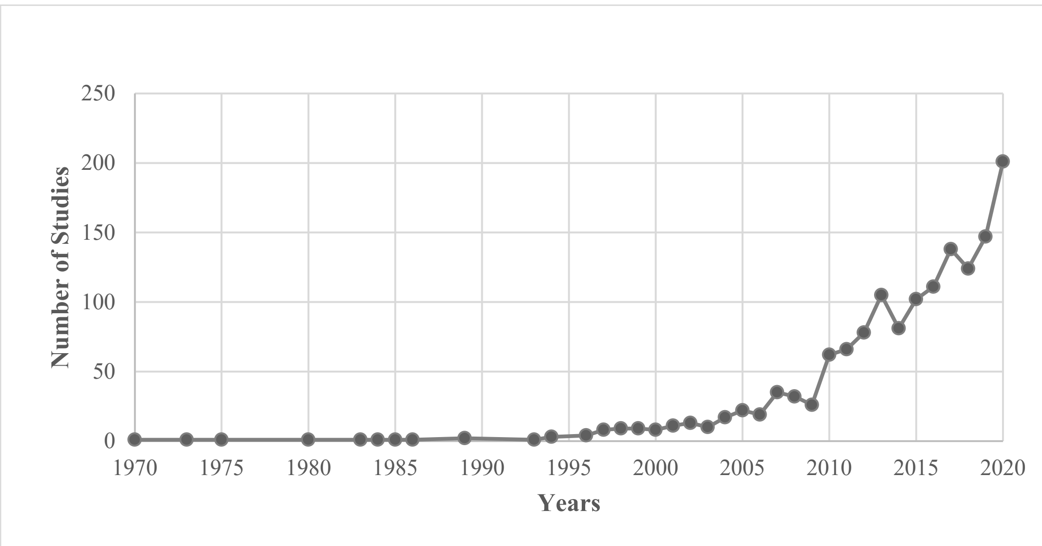

Figure 1. Number of subclinical mastitis studies in water buffaloes by year.

Table 1. Characteristics of buffalo-based studies that met the inclusion criteria.

Study no.	Authors	Years	N	M	Herd size
1	Alacam et al., 1989	1989	256	25	Medium
2	Bachaya et al., 2005	2005	300	234	Medium
3	Dhakal, 2006	2006	60	13	Small
4	Chishty et al., 2007	2007	370	145	Large
5	Sharif and Ahmad, 2007	2007	100	51	Small
6	Sharma et al., 2007	2007	500	360	Large
7	Ali et al., 2011	2011	600	264	Large
8	Raza et al., 2011 1	2011	300	165	Medium
9	Salvador et al., 2011	2011	205	95	Medium
10	Shahid et al., 2011	2011	25	20	Small
11	Guha et al., 2012 a	2012	514	271	Large
	Guha et al., 2012 b		615	152	
12	Hameed et al., 2012	2012	382	139	Large
13	Mustafa et al., 2012	2012	272	34	Medium
14	Hussain et al., 2013	2013	592	90	Large
15	Mustafa et al., 2013	2013	272	34	Medium
16	Pankaj et al., 2013	2013	82	24	Small
17	Sadashiv and Kaliwal, 2013 a	2013	46	20	Small
	Sadashiv and Kaliwal, 2013 b		102	63	Medium
18	Shahzad et al., 2013	2013	164	18	Medium
19	Charaya et al., 2013	2013	66	19	Small
20	Hamed and Zaitoun, 2014	2014	239	66	Medium
21	Ali et al., 2015	2015	48	15	Small
22	Arfan et al., 2016	2016	150	49	Medium
23	Baloch et al., 2016	2016	210	114	Medium
24	Hardenberg, 2016	2016	28	8	Small
25	Jhambh et al., 2017	2017	217	69	Medium
26	Swami et al., 2017	2017	60	17	Small
27	Baloch et al., 2018	2018	423	114	Large
28	Hussain et al., 2018	2018	1036	402	Large
29	Tanmay et al., 2018	2018	1650	685	Large
30	Kashyap et al., 2019	2019	120	82	Medium
31	Patel et al., 2019	2019	92	21	Small
32	Koldas Urer et al., 2019	2019	200	48	Medium
33	Chhabra et al., 2020	2020	102	30	Medium
34	Hussain et al., 2020	2020	598	332	Large

N = Total number of buffaloes, M = Number of buffaloes with subclinical mastitis

calculate variance (τ^2). Egger's linear regression test and Duval and Tweedie's trim and fill method was used to detect the publication biases in the samples and funnel plots were drawn. In order to determine the source of heterogeneity between studies, covariates such as the study year range (1989–2001, 2002–2011 and 2012–2020) and herd size [small (≤100 heads), medium (101–300 heads), large (>300 heads)] were created and subgroup analyses

Table 2. Characteristics of udder quarter-based studies that met the inclusion criteria.

Study no.	Authors	Years	N	M	Herd size
1	Memon et al., 1999	1999	378	110	Small
2	Ahmad, 2001	2001	2340	157	Large
3	Khan et al., 2004	2004	172	54	Small
4	Khan and Muhammed, 2005	2005	200	54	Small
5	Bachaya et al., 2005	2005	1200	705	Medium
6	Bulla et al., 2006	2006	239	1	Small
7	Sharma et al., 2007	2007	2000	900	Large
8	Sharma and Sindhu, 2007	2007	5707	1878	Large
9	Sharif and Ahmad, 2007	2007	400	151	Small
10	Ozenc et al., 2008	2008	1637	206	Large
11	Ali, 2009	2009	300	183	Small
12	Muhammed et al., 2010	2010	400	118	Medium
13	Guha et al., 2012 a	2012	2048	496	Large
	Guha et al., 2012 b		2452	508	
14	Beheshti et al., 2011	2011	201	55	Small
15	Raza et al., 2011 1	2011	1200	521	Medium
16	Hameed et al., 2012	2012	1384	222	Large
17	Hussain et al., 2013	2013	2202	140	Large
18	Pankaj et al., 2013	2013	326	38	Small
19	Charaya et al., 2013	2013	262	48	Small
20	Sadashiv and Kaliwal, 2013 1	2013	592	113	Medium
21	Ali et al., 2014	2014	1465	612	Large
22	Preethirani et al., 2015	2015	190	86	Small
23	Hardenberg, 2016	2016	104	11	Small
24	Baloch et al., 2016	2016	840	330	Medium
25	Swami et al., 2017	2017	240	35	Small
26	Jhambh et al., 2017	2017	864	148	Medium
27	Tanmay et al., 2018	2018	6460	2675	Large
28	Sharma et al.,2018	2018	4452	1503	Large
29	Yigit et al., 2018	2018	167	95	Small
30	Ahmed et al., 2018	2018	682	302	Medium
31	Ozenc et al., 2019	2019	475	84	Small
32	Hussain et al., 2020	2020	2392	449	Large
33	Chhabra et al., 2020	2020	401	73	Medium

N = Total number of udder quarters, M = Number of udder quarters with subclinical mastitis

were made according to these covariates. Differences in subclinical mastitis prevalence among subgroups were analyzed by univariate metaregression analysis. Subgroup and metaregression analyses by years were performed for 2002–2011 and 2012–2020 year groups, as there were not enough studies to create a subgroup between 1989 and 2001. The significance level of Cochran Q heterogeneity statistics was accepted as $p < 0.10$, and the significance level of effect sizes and coefficients was accepted as $p < 0.05$. Statistical analyses were performed using the "meta", "tidyverse", and "metaphor" packages in R (www.r-project.org/). Within the scope of the study, flow diagram of the

Table 3. Characteristics of subclinical mastitis studies with agent identification.

Study no.	Authors	Years	N	E	Herd size	Agents
1	Memon et al., 1999 a	1999	110	12	Small	*Klebsiella pneumonia*
	Memon et al., 1999 b			51		*Staphylococcus* spp.
	Memon et al., 1999 c			23		*Streptococcus* spp.
	Memon et al., 1999 d			12		*E. coli*
2	Ozenc et al., 2008 a	2008	206	1	Large	*Bacillus* spp.
	Ozenc et al., 2008 b			20		*Staphylococcus* spp.
	Ozenc et al., 2008 c			11		*CNS*
3	Ali et al., 2011 a	2011	234	29	Large	*Bacillus* spp.
	Ali et al.,2011 b			12		*Klebsiella pneumonia*
	Ali et al., 2011 c			16		*Corynebacterium* spp.
	Ali et al., 2011 d			66		*Staphylococcus* spp.
	Ali et al., 2011 e			18		*Streptococcus* spp.
	Ali et al., 2011 f			38		*E. coli*
4	Beheshti et al., 2011 a	2011	173	14	Small	*Corynebacterium* spp.
	Beheshti et al., 2011 b			90		*Staphylococcus* spp.
5	Charaya et al., 2013 a	2013	50	32	Small	*Staphylococcus* spp.
	Charaya et al., 2013 b			18		*Streptococcus* spp.
6	Pankaj et al., 2013 a	2013	44	16	Small	*Streptococcus* spp.
	Pankaj et al., 2013 b			21		*Coagulase negative staphylococci*
7	Ali et al., 2015 a	2015	15	9	Small	*Staphylococcus* spp.
	Ali et al., 2015 b			4		*Streptococcus* spp.
	Ali et al., 2015 c			2		*E. coli*
8	Preethirani et al., 2015 a	2015	86	6	Small	*Staphylococcus* spp.
	Preethirani et al., 2015 b			16		*Streptococcus* spp.
	Preethirani et al., 2015 c			56		*CNS*
	Preethirani et al., 2015 d			8		*E. coli*
9	Jhambh et al., 2017 a	2017	75	10	Medium	*Staphylococcus* spp.
	Jhambh et al., 2017 b			26		*Streptococcus* spp.
	Jhambh et al., 2017 c			36		*CNS*
10	Sharma et al.,2018 a	2018	1649	5	Large	*Bacillus* spp.
	Sharma et al.,2018 b			5		*Klebsiella pneumonia*
	Sharma et al.,2018 c			10		*Corynebacterium* spp.
	Sharma et al.,2018 d			853		*Staphylococcus* spp.
	Sharma et al.,2018 e			622		*Streptococcus* spp.
11	Ozenc et al., 2019 a	2019	84	5	Small	*Bacillus* spp.
	Ozenc et al., 2019 b			13		*Staphylococcus* spp.
	Ozenc et al., 2019 c			6		*Streptococcus* spp.
	Ozenc et al., 2019 d			23		*CNS*
	Ozenc et al., 2019 e			4		*E. coli*
12	Patel et al., 2019 a	2019	21	1	Small	*Bacillus* spp.
	Patel et al., 2019 b			2		*Staphylococcus* spp.
13	Chhabra et al., 2020 a	2020	96	61	Medium	*Staphylococcus* spp.
	Chhabra et al., 2020 b			35		*Streptococcus* spp.
	Chhabra et al., 2020 c			145		*E. coli*
14	Hussain et al., 2020	2020	449	257	Large	*Staphylococcus* spp.

*N: Total number of isolates, A: Number of agents literature search selection for metaanalysis was applied with reference to the PRISMA 2020 checklist (Figure 2) [13].

3. Results

A high level of heterogeneity was found between both water buffalo-based and udder quarter-based studies in metaanalyses (Cochran's Q = 1916.73, $p < 0.001$, $I^2 = 98.17$; Cochran's Q = 8289.38, $p < 0.001$, $I^2 = 99.61$, respectively). According to the DerSimonian-Laird method used to determine the variance (τ^2) between studies, $\tau^2 = 0.035$ was found in water buffalo-based studies and $\tau^2 = 0.027$ was found in udder quarter-based studies. The pooled prevalence of subclinical mastitis was 0.38 in water buffalo-based studies (95% CI: 0.32–0.44); and 0.28 (95% CI: 0.23–0.34) in udder quarter-based studies (Table 4).

The forest plots are created as a result of the metaanalysis in studies based on water buffalo and udder quarters. The heterogeneities among the studies can be seen in the forest plots (Figure 3).

According to the results of Egger's linear regression test which was performed to detect publication bias in the sample, there was no publication bias in water buffalo-based studies ($p = 0.452$), but there was publication bias in udder quarter-based studies ($p = 0.010$) (Table 5). These publication biases are shown in the funnel plots (Figure 4).

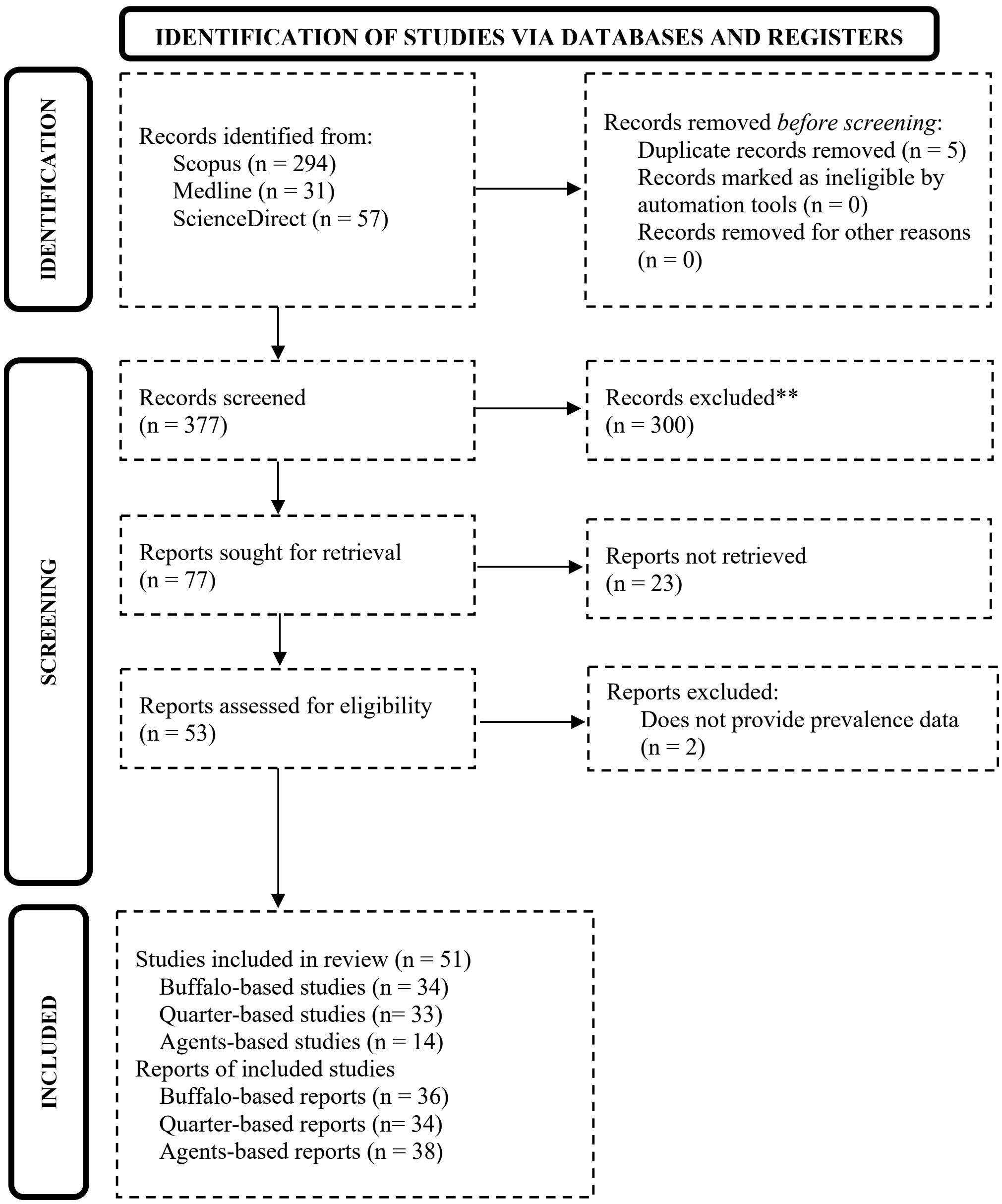

Figure 2. Flow diagram of the literature search selection for studies.

Table 4. Metaanalysis results of buffalo-based and udder quarter-based studies.

	N	Prevalence (95 % CI)	Cochran's Q	I^2	τ^2	p (Cochran's Q)
Buffalo-based	36	0.38 (0.32–0.44)	1916.73	98.17	0.035	< 0.001
Udder quarter-based	34	0.28 (0.23–0.34)	8289.38	99.61	0.027	< 0.001

CI: Confidence interval, I^2: Ratio of variance in observed effects to variance in true effects rather than sampling error, τ^2: Variance in true effect sizes

In order to eliminate the publication bias in the udder quarter-based study sample, 16 virtual studies were included in the analysis hypothetically using Duval and Tweedie's trim and fill method. As a result of the analysis, the adjusted pooled prevalence of udder quarter-based studies was calculated as 0.12 (95% CI: 0.57–0.18). Thus, the variance between studies was increased from $\tau^2 = 0.027$ to $\tau^2 = 0.051$. In addition, I^2 (%) = 99.8 was calculated and the rate at which the observed effect sizes reflected the actual effect size increased.

As a result of the subgroup analyses made according to years, the pooled prevalence values between 2002 and 2011 and between 2012 and 2020 were 0.54 and 0.33, respectively, in water buffalo-based studies and 0.36 and 0.25, respectively, in udder quarter-based studies. As a result of the subgroup analyses made according to herd sizes, the pooled prevalence of subclinical mastitis in large, medium-sized, and small businesses were 0.41, 0.39, and 0.36, respectively in water buffalo-based studies and 0.27, 0.34, and 0.28, respectively, in udder quarter-based studies (Table 6).

In water buffalo-based studies, the difference in subclinical mastitis prevalence among year subgroups was found to be statistically significant ($p = 0.002$); however, it was not found to be significant in udder quarter-based studies ($p = 0.182$). There was no statistically significant difference between herd size groups in either water buffalo or udder quarter-based studies ($p = 0.791$; $p = 0.600$, respectively).

In the univariate metaregression analyses, in the water buffalo-based studies the effect of year groups on the prevalence of subclinical mastitis was found to be statistically significant ($p = 0.001$) but the effect of herd size was found to be insignificant ($p = 0.867$) while in studies based on udder quarters, the effects of both the year groups and herd size were found to be insignificant ($p = 0.053$; $p = 0.589$, respectively) (Table 7). In the univariate metaregression models created, the hypothesis that the true variances of the studies were zero was rejected, and it was concluded that the actual effect sizes varied between studies ($p < 0.001$). I^2 (%) statistics calculated according to year groups and herd sizes were found to be 97.75% and 98.07% in water buffalo-based studies, and 99.44% and 99.49% in udder quarter-based studies, respectively. These results have shown that the variance of the observed effects according to the regression line is due to the variance in the real effects, not the sampling error at the specified rates.

In the second stage of the study, metaanalyses were performed separately according to the factors determined in 38 prevalence data obtained from 14 studies, in which subclinical mastitis prevalence were calculated and agent identification was performed, and pooled prevalence were calculated. The pooled prevalence for *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., CNS, and *E. coli* identified in the isolates obtained from water buffaloes with subclinical mastitis were calculated as 0.36, 0.26, 0.03, 0.39, and 0.11, respectively (Table 8).

According to the publication bias test results belonging to agent prevalences, publication bias was detected only in studies that had samples with *Bacillus* spp. and CNS isolates ($p < 0.001$ and $p = 0.042$, respectively). The revised common prevalence values obtained by adding 3 studies to each of the analyses alongside the samples with *Bacillus* spp. and CNS isolates using Duval and Tweedie's trim and fill method in order to eliminate the publication bias in studies were respectively calculated as 0.004 and 0.097 (Table 9).

4. Discussion

Since their first domestication, water buffaloes have been of great importance for humankind at both micro- and macroeconomic levels. Water buffaloes, alongside being an efficient source for milk and dairy products, horns and leather for centuries, have also been utilized as running draught animals. Despite generally being fed low quality and inexpensive feed, they have had the potential to produce higher quality milk and meat than cattle [14].

Water buffaloes are quite resistant to diseases taking into consideration the adverse environmental conditions they live in. The effects of many diseases dangerous for cattle, such as trypanosomiasis, tuberculosis, brucellosis, rinderpest, and piroplasmosis, are generally to a lesser degree in water buffaloes [15]. However, mastitis, which results in a decrease in milk yield and causes serious economic losses, is an important disease for water buffaloes also.

Numerous studies with small sample sizes were found for subclinical mastitis in water buffalo farming. As the number of scientific studies on this subject has increased,

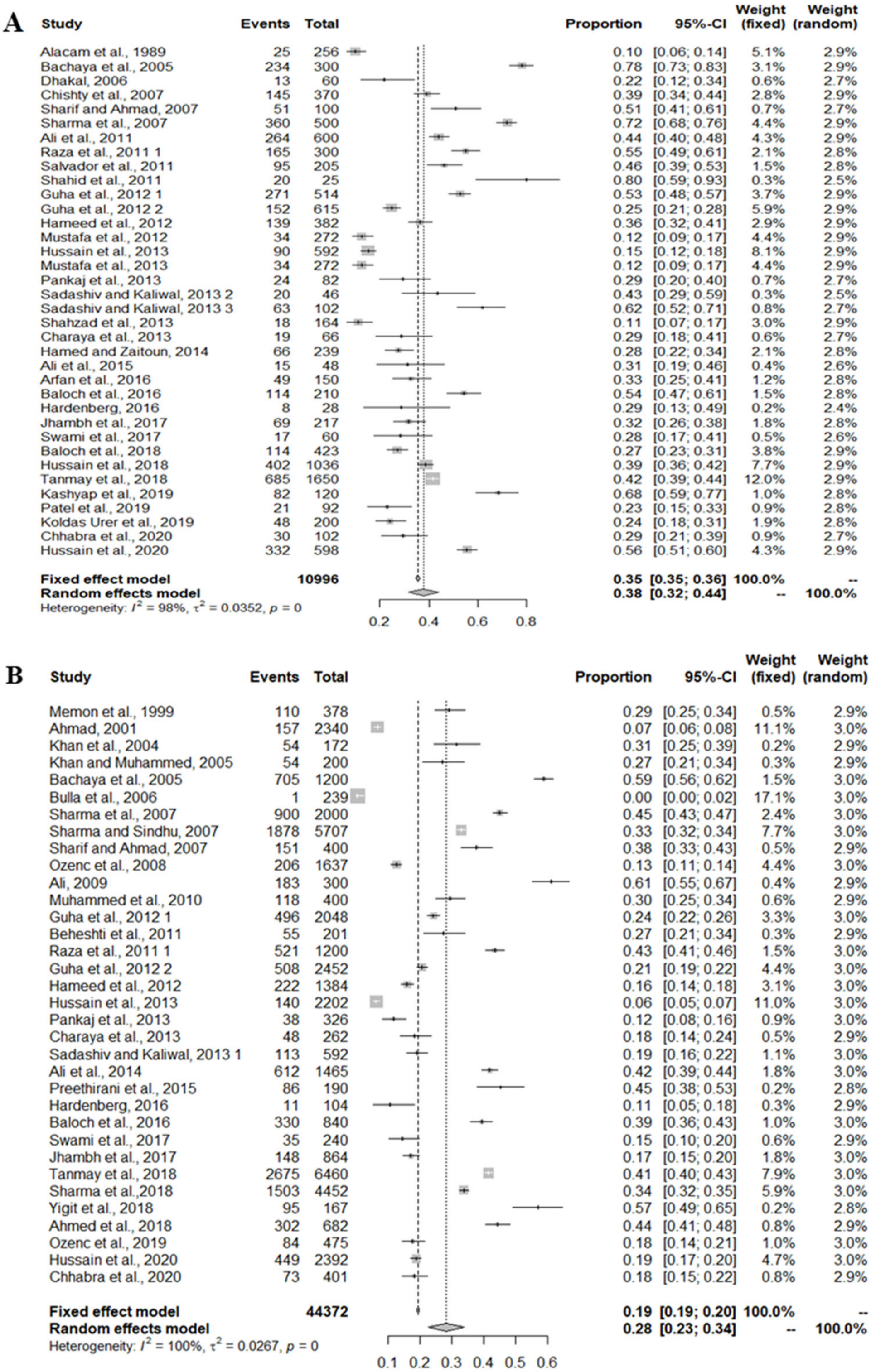

Figure 3. Forest plots of metaanalyses of subclinical mastitis prevalences buffalo-based (A) and udder quarter-based (B) studies.

there was born a need for more accurate, reliable results with reduced risk of bias. With the metaanalysis, the results of multiple independent studies on this subject were synthesized and the opportunity to make statistical analysis of the research findings and to reinterpret them was provided. It has been reported that the common effect

Table 5. Publication bias test of buffalo-based and udder quarter-based study samples.

Egger's linear regression test			
	Coefficient	t statistic	p-value
Buffalo-based	2.175	0.76	0.452
Udder quarter-based	12.375	2.75	0.010

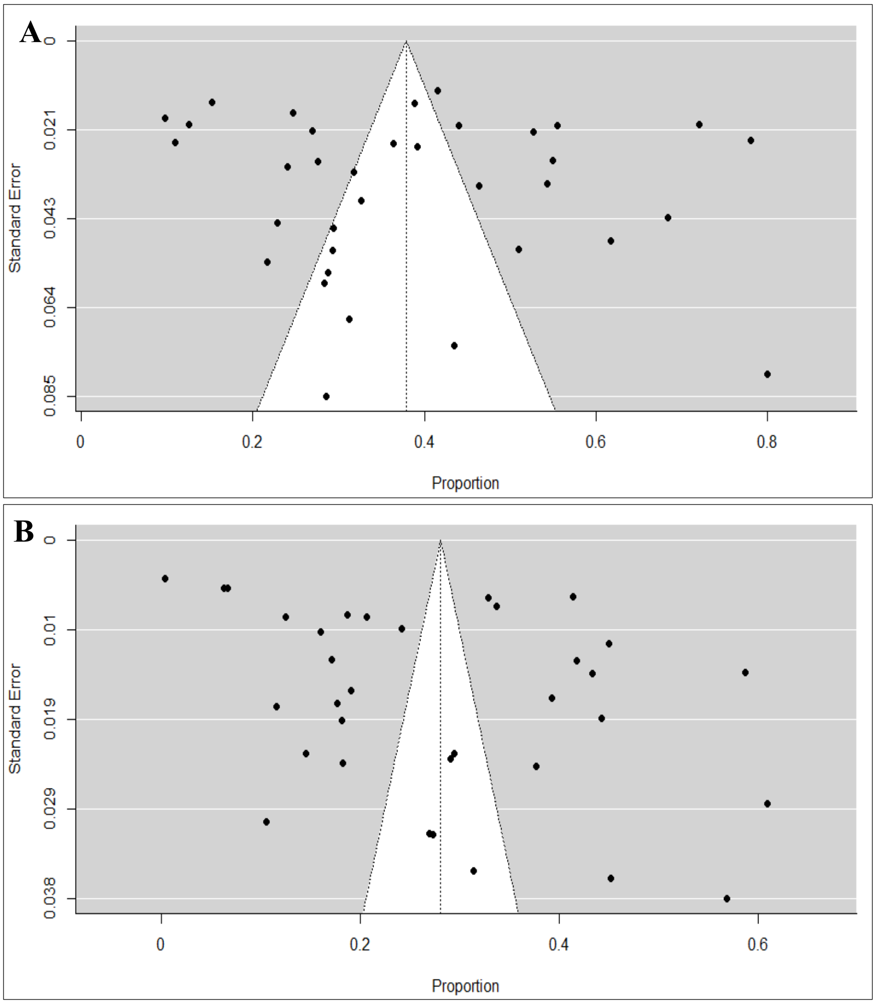

Figure 4. Funnel plots of buffalo-based (A) and udder quarter-based (B) studies.

size obtained as a result of the metaanalysis can increase the validity of individual studies with similar findings or the reasons for the differences between individual studies can be investigated to establish new hypotheses and obtain new information [16, 17].

It was determined that the prevalence values calculated in studies on subclinical mastitis in water buffaloes have a high variation, and their prevalence in water buffalo-based studies has a wide distribution range between 9.8% and 80%. As a result of the metaanalysis in this study, the pooled prevalence of subclinical mastitis in water buffaloes was calculated as 38% in water buffalo-based studies.

In many water buffalo-based studies, subclinical mastitis prevalence results were found within the confidence interval (32%–44%) of the pooled prevalence calculated as a result of the metaanalysis [18-23]. The prevalence value was found to be lower than the lower limit of the confidence interval in some studies [24-26],

Table 6. Results of subgroup analysis of buffalo and quarter-based studies.

	N	Prevalence	Cochran's Q	I^2	τ^2
Buffalo-based					
Years					
2002–2011	9	0.54 (0.42–0.66)	286.44	97.2	0.030
2012–2020	26	0.33 (0.27–0.39)	894.16	97.2	0.022
Between groups	Cochran's Q = 9.63, df = 1, p = 0.002				
Herd-size					
Large	11	0.41 (0.31–0.50)	745.05	98.7	0.025
Medium	14	0.39 (0.26–0.51)	873.87	98.5	0.057
Small	10	0.36 (0.26–0.46)	61.46	85.4	0.020
Between groups	Cochran's Q = 0.47, df = 2, p = 0.791				
Quarter-based					
Years					
2002–2011	11	0.36 (0.21–0.51)	4427.48	99.8	0.062
2012–2020	21	0.25 (0.19–0.31)	3063.98	99.3	0.018
Between groups	Cochran's Q = 1.78, df = 1, p = 0.182				
Herd size					
Large	11	0.27 (0.17–0.36)	3208.39	99.7	0.021
Medium	8	0.34 (0.22–0.45)	715.17	99.0	0.027
Small	13	0.28 (0.17–0.38)	1265.23	99.1	0.039
Between groups	Cochran's Q = 1.02, df = 2, p = 0.600				

df = Degree of freedom, CI: 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

and higher than the upper limit of the confidence interval in some studies [21, 25, 27].

In udder quarter-based studies, the prevalence of subclinical mastitis was also found to have a wide distribution range between 0.4% and 61%. The pooled prevalence of subclinical mastitis was calculated as 28% in udder quarter-based studies. In many udder quarter-based studies, subclinical mastitis prevalence results were found within the confidence interval (23%–34%) of the pooled prevalence value we calculated as a result of the metaanalysis [28-35]. The prevalence value was found to be lower than the lower limit of the confidence interval in some studies [21, 26, 36] and higher than the upper limit of the confidence interval in some studies [35, 37, 38].

As a result of the metaanalysis conducted in this study, the pooled subclinical mastitis prevalence of water buffalo-based studies was calculated to be approximately 25% lower than the pooled prevalence of udder quarter-based studies. Similarly, in some of the individual studies, it has been reported that the prevalence of subclinical mastitis is lower in water buffalo-based studies than in udder quarter-based studies [34, 39, 40].

It has been reported that water buffaloes are well adapted to humid environments, have lower body temperatures, have slower respiratory and pulse rates, are more resistant to diseases, and have a low prevalence of subclinical mastitis due to their nipple anatomy [1]. However, some studies also report that the prevalence of mastitis is similar in cows and water buffaloes [37, 41]. In metaanalysis studies on the prevalence of subclinical mastitis in cows, the pooled prevalence was reported by Krishnamoorthy et al. [42] as 41% and by Getaneh and Gebremedhin [43] as 37%. Similarly, in a metaanalysis study by Krishnamoorthy et al. [44], the prevalence of subclinical mastitis was calculated as 42% in dairy cows and 46% in water buffaloes. As a result of the metaanalysis conducted in this study, the pooled prevalence of subclinical mastitis was found to be lower in water buffaloes.

According to the results of subgroup and metaregression analyses performed by year (2002–2011 and 2012–2020) and herd size (large, medium, small) for water buffalo and udder quarter-based metaanalysis, there was significant difference in subclinical mastitis prevalence among year subgroups in only water buffalo-

Table 7. Results of univariate metaregression analysis of buffalo- and udder quarter-based studies.

	Coefficient (95% CI)	SE	z statistic	p-value
Buffalo-based				
Years				
Intercept	0.334 (0.271–0.396)	0.032	10.475	<0.001
2002–2011	0.206 (0.082–0.329)	0.063	3.269	0.001
2012–2020 (reference)	0.00	-	-	-
Model test: Q(df = 1) = 10.684, p = 0.001				
Goodness of Fit: Q(df = 33) = 1180.594, p < 0.001				
R^2 Analog = 0.01, I^2 (%)= 97.75				
Herd Size				
Intercept	0.406 (0.297–0.516)	0.056	7.261	<0.001
Medium	–0.018 (–0.165 to 0.129)	0.075	–0.243	0.808
Small	–0.044 (–0.208 to 0.119)	0.083	–0.534	0.593
Large (reference)	0.00	-	-	-
Model test: Q(df = 2) = 0.29, p = 0.867				
Goodness of Fit: Q(df = 32) = 1680.37, p < 0.001				
R^2 Analog = 0.05, I^2 (%)= 98.07				
Quarter-Based				
Intercept	0.251 (0.187–0.315)	0.033	7.68	<0.001
2002–2011	0.108 (–0.002 to 0.217)	0.056	1.93	0.053
2012–2020 (reference)	0.00	-	-	-
Model test: Q(df = 1) = 3.74, p = 0.053				
Goodness of Fit: Q(df = 30) =7491.46, p < 0.001				
R^2 Analog = 0.01, I^2 (%)=99.44				
Herd Size				
Intercept	0.267 (0.174–0.360)	0.05	5.61	<0.001
Medium	0.070 (–0.074 to 0.214)	0.07	0.96	0.338
Small	0.008 (–0.119 to 0.136)	0.07	0.12	0.901
Large (reference)	0.00	-	-	-
Model test: Q(df = 2) = 1.06, p = 0.589				
Goodness of fit: Q(df = 29) = 5188.80, p < 0.001				
R^2 analog = 0.001, I^2 (%)=99.49				

Df = Degree of freedom, CI: 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

based studies. While subclinical mastitis has decreased by approximately one third in recent years according to water buffalo-based studies, the effect of herd size was not found to be significant. In studies based on udder quarters, it was observed that neither herd sizes nor the year groups have a significant effect on the prevalence of subclinical mastitis ($p > 0.05$). This may be due to the lack of sufficient literature on the classification of buffalo herd sizes and the fact that buffalo milk businesses are mostly small scale enterprises. In addition, it has come to light that due to the lack of full implementation of modern approaches in water buffalo farming, significant improvements and developments could not be achieved in health protection areas so far. However, although there was no significant difference in subclinical mastitis prevalence between years, it was observed that the prevalence of the disease decreased by 39% based on water buffalo-based studies and by 31% based on udder quarter-based studies.

Table 8. Pooled prevalence results of some bacterial species in isolates from buffaloes with subclinical mastitis.

Agents	k	Prevalence (95% CI)	Cochran Q	I^2 index	τ^2 statistic	p-value (Cochran Q)
Staphylococcus spp.	13	0.36 (0.24–0.49)	672.349	98.22	0.050	<0.001
Streptococcus spp.	10	0.26 (0.15–0.37)	267.946	96.64	0.027	<0.001
Bacillus spp.	5	0.03 (0.01–0.05)	37.021	89.20	<0.001	<0.001
CNS	5	0.39 (0.12–0.65)	191.207	97.91	0.086	<0.001
E. coli	5	0.11 (0.06–0.15)	12.012	66.70	0.002	0.017

k: Number of studies, CI: Confidence interval, 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 9. Publication bias tests of agent prevalences in study samples.

Agent	Egger's linear regression test		Duval and Tweedie's trim and fill test		
	z statistic	p-value	Prevalence (95% CI)	Cochran Q	p-value (Cochran Q)
Staphylococcus spp.	0.988	0.323	-	-	-
Streptococcus spp.	0.734	0.463	-	-	-
Bacillus spp.	3.391	<0.001	0.004 (0.001–0.028)	74.36	<0.001
CNS	2.030	0.042	0.097 (0.001–0.343)	427.09	<0.001
E. coli	0.212	0.832		-	-

In the subclinical mastitis prevalence studies in which agent identification was applied, the highest rated mastitis agents were CNS with 39% and *Staphylococcus* spp. with 36% and the lowest rated was *Bacillus* spp. mastitis with 3%. Krishnamoorthy et al. [42], in their study, calculated the prevalences for *Staphylococcus* spp., *Streptococcus* spp. and *E. coli*-induced subclinical mastitis as 45%, 13%, and 14%, respectively. Compared to our present study's findings, these numbers show a higher rate for *Staphylococcus* spp. mastitis, a lower rate for *Streptococcus* spp. mastitis, and similar rates for *E. coli* mastitis.

This study provided a stronger estimate of the prevalence of subclinical mastitis in water buffaloes by eliminating the inconsistencies of the effect size in the population in individual studies. Although there are some criticisms made towards metaanalysis, the fact that it makes a stronger and more precise estimation of the population effect size provides the opportunity to work with a large sample by combining small-scale individual studies, and thus allows for the elimination of inconsistencies and has deemed this method increasingly valuable and more frequently used day by day. However, in order to be able to obtain the correct results from this practice, systematically and carefully selecting and examining the studies that will be included in the analysis, using the appropriate statistical model, and interpreting the results of the analysis correctly are necessary.

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