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Evidence of mastitis in buffalo in Iraq: A systematic review and meta-analysis

Hussien W. Ali, Mohammad O. Dahl*

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

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***Correspondence:**

Corresponding author: Mohammad O. Dahl
E-mail address: mdahl@uomosul.edu.iq

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ABSTRACT

This study was conducted to identify the evidence and knowledge gaps in studies investigated mastitis and quantify the prevalence of clinical mastitis (CM) and subclinical mastitis (SCM) in buffalo in Iraq. Databases used as search engines to track the studies included PubMed, CABI, IASJ, and Google Scholar. Additional pertinent studies identified via other methods were also considered for inclusion. Identified studies were divided into two main groups: (i) studies reported proportion of mastitis cases in lactating buffaloes, which were considered for meta-analysis; and (ii) studies that investigated presence of bacteria in milk sampled from buffaloes with different status of mastitis. A total of 17 studies were qualified for review: 9 reported proportion of mastitis episodes, and 13 isolated pathogenic bacteria in lactating buffalo milk. Prevalence of CM and SCM was 20.89% and 29.62%, respectively. The most isolated pathogens were *Staphylococcus* spp. (46.75%) followed by *Streptococcus* spp. and other miscellaneous bacteria at 32.96%, 19.04%, respectively. A substantial risk of bias was revealed in most of the identified studies. In conclusion, it is important to conduct further studies that apply standard epidemiological tools for estimating incidence and identifying risk factors of CM and SCM in buffaloes in Iraq.

Introduction

Mastitis is simply defined as an inflammation of the mammary gland caused by different infectious agents and characterized by physical and/or chemical and pathological changes in milk accompanied or not by systemic reactions (Constable *et al.*, 2017). Although multi-pathogens can cause mastitis, bacteria are considered the most common causative agents (Purohit *et al.*, 2014). Risk of mastitis is increased during the periparturient period, with the greatest incidence rate during the first month of the lactation period (Waller, 2000). However, the susceptibility of buffaloes to mastitis is considered lower than that of cows (Wanasinghe, 1985), which is attributed to the anatomical characteristics of the udder in buffaloes that minimize the opportunities of microorganisms' invasion (Purohit *et al.*, 2014). Such characteristics include long and narrow teat canal, thick keratinized epithelial layer of the streak canal, and tightly closed teat orifice (Sharma *et al.*, 2012). Finally, the more alkaline property of buffalo milk compared to cow milk can also contribute to decreasing the risk of mastitis in buffalo (Briñez *et al.*, 2000).

Mastitis is considered the costliest disease faces dairy producers (Heikkilä *et al.*, 2012). Buffalo is the second most essential species for dairy production after cow, and the price of buffalo milk can reach twice that of cow (IDF Bulletin 2013; Pisanu *et al.*, 2019). In Iraq, buffalo is mainly raised for production of milk and its products, particularly the thickened cream, which is traditionally called "Qaymar" or "Geymar" and mostly eaten during the holidays although its high price. Additionally, buffalo milk is used for yogurt and ice-cream production. According to the Agricultural Statistical Atlas (2011), the total number of buffalo in Iraq is estimated at 285,437 head, mostly found around rivers and marshy areas.

Mastitis has been investigated in buffalo in Iraq. In a very first detailed investigation, 151 buffaloes from Baghdad and Missan governorates showed that the incidence of clinical mastitis (CM) and subclinical mastitis (SCM) was 25% and 31.9%, respectively (Abdul-Razak, 1982). In that study, the most isolated bacteria were staphylococci followed by *Streptococci*. In another study, 13.5% and 16% of buffaloes in Nineveh were affected with CM and SCM, respectively (Al-Kass, 2005). Several other studies were performed in different Iraqi governorates. However, a systematic review that examines current evidence and identifies knowledge gaps for those studies was not available. In addition, a meta-analysis that can numerically summarize the current evidence of mastitis in buffalo in Iraq has not been performed. Therefore, the objective of this systematic review and meta-analysis was to identify the evidence and knowledge gaps in studies investigated mastitis in buffalo in Iraq, and numerically quantify the identified current evidence.

Materials and methods

Systematic Review

The current systematic review and meta-analysis were performed according to PRISMA 2020 statement (Page *et al.*, 2021).

Eligibility Criteria

Studies considered for inclusion in this review included studies reported original data, published in peer-reviewed journals, and written in Arabic or English. In addition, MSc thesis and PhD dissertations were also eligible for inclusion in this review because this type of research was evaluated as that of studies published in peer-reviewed journals (Cook *et al.*, 1993). On the other hand, narrative reviews and studies that did not present original data were excluded. In this review process, studies were grouped on a basis of those studies investigated mastitis in buffalo in Iraq before the date of this review, whether they reported the proportions of

CM and/or SCM or isolated the causative microorganisms. In those studies, mastitis was considered as physical and/or chemical and pathological changes in milk detected clinically and/or by direct or indirect tests used for subclinical cases.

Information sources

Scientific search engines used for tracking the pertinent peer-reviewed reports included: (i) PubMed; the database of the United States National Library of Medicine (MEDLINE) at the National Institutes of Health as well as life science journals and books (https://pubmed.ncbi.nlm.nih.gov/), (ii) CABI (Centre for Agriculture and Bioscience International); a UK-based database (https://www.cabi.org/), (iii) IASJ (Iraqi Academic Scientific Journals database; https://www.iasj.net/), and (iv) Google Scholar (https://scholar.google.com/). Google Scholar can track more local studies than other academic search engines (Dahl, 2020). In addition, Google Scholar is considered a powerful tool and can be used with other academic search engines for evidence reviews (Haddaway *et al*., 2015). The additional sources used to identify relevant studies included reference list of identified articles and local colleagues who worked on pertinent topics. Finally, the search was performed on August 24, 2021.

Search strategy

In this review, the words buffalo, mastitis, and Iraq have been identified by both authors as keywords that can retrieve as much as possible of pertinent studies in each search engine used for this purpose. Additional words such as clinical, subclinical, and water for water buffalo did not add value to the retrieved records; therefore, they were removed. The keywords were entered in the search box including a comma and one space after each word as the following: buffalo, mastitis, Iraq. Double quotations marks (" ") were not used because the exact phrase was not intended. In this search, the operators "and" and "or" as Boolean operators between the selected keywords were removed because they did not add value to the retrieved records. In addition, although commas did not change the retrieved records number, they were added between the keywords just to make it easier to view them. Finally, automatic filtering was not applied to any of used search engines.

Selection Process

Both authors together reviewed the retrieved records to identify relevant studies. In this process, duplicated records were identified and removed manually, as no automation filters have been applied. After the final list of qualified studies was specified from the search engines, the references list of each identified article has been screened by both authors together, too. In this process, already identified studies were omitted, while relevant studies were added to the list of qualified studies.

Data collection and items

Both authors worked together to manually extract the data from the qualified studies. Study-level data included: year and area of the study conduct, number of buffaloes/samples investigated, number (or proportion) of animals with mastitis, type of mastitis (CM, SCM), tests used for diagnosis of SCM, isolated bacteria, number of isolates, and method used for detection of bacteria. In this review, when the number of animals affected with mastitis (or number of isolates) was not mentioned in a specific study, the number was calculated from provided proportion and total number of investigated animals or samples.

Assessing risk of bias in identified studies

In this study, both authors assigned a checklist to assess risk of bias according to criteria adapted from different critical appraisal tools including STORBE checklist (Vandenbroucke *et al*., 2014), Hoy *et al*.'s risk of tool (Hoy *et al*., 2012), and JBI checklists for both prevalence studies (Munn *et al*., 2015) and analytical cross-sectional studies (Moola *et al*., 2020). The current checklist was assigned because the majority of existing appraisal tools focused on analytical studies, whereas the identified studies in the current review were mostly descriptive, although a checklist for descriptive studies is available. The assigned criteria were four items threatening external validity (included: (i) was the sample size calculated and justified, (ii) were inclusion and exclusion criteria clearly defined, (iii) were study animals randomly selected, and (v) were study animals close to represent target population), and four items threatening internal validity (included (i) were study animals clearly described in details, (ii) was the outcome of interest clearly defined, (iii) were standard criteria used to measure the outcome of interest, and (v) was data appropriately analyzed including clear calculation to the outcome of interest calculation). The scoring was based on existence of the criterion in the evaluated study as the following: "Yes", i.e., the criterion is exist, worth 0, i.e., no risk of bias, "Somewhat" worth 50, "Not clear" worth 75, and "No", i.e., the criterion is not exist, worth 100, i.e., presence of bias. For each study, percentages of risk of bias threatening the external and internal validity as well as the overall risk of bias were calculated (representing the mean of scores given to each criterion). Finally, the authors independently assessed the studies manually without any automation tool, and a consensus was used to resolve any disagreement in the assessment.

Meta-analysis

Studies qualified for review with clear and accurate calculations for the proportion of buffaloes reported as affected with mastitis were included in an analysis estimated the prevalence of mastitis. On the other hand, studies qualified for review with clear and accurate calculations for the proportion of a specific pathogen isolated from buffaloes affected with mastitis were included in another analysis estimated the prevalence of pathogens in mastitis buffaloes. In both analyses, random-effects model was applied based on the assumption that the included studies had true variation in the effect size (Borenstein *et al*., 2010). That is, estimation of prevalence of mastitis included studies reported CM or SCM with different methods for detection of SCM. In addition, estimation of prevalence of pathogens included studies isolated different pathogens by use of different media. In both analyses, evidence of statistical heterogeneity was assessed by Cochran's Q test, and the variability as a function of heterogeneity rather than chance was estimated by I2 statistic (Higgins *et al*., 2003). In addition, potential presence of small-study effect (i.e., evidence of bias) was examined by Egger test; a regression-based test for continuous data (Egger *et al*., 1997). In all analyses, P-value of ≤ 0.05 was considered significant. Finally, the data was analyzed using STATA 13.0 (StataCrop., College Station, TX, USA) applying the command "metaprop" with the option "by" to generate subgroup estimates, and the command "metabias" with the option "egger" for Egger regression.

Results

Systematic review

Both PubMed and CABI search engines revealed zero reports, whereas IASJ and Google Scholar identified 3 and 780 records, respectively (Fig. 1). A total of 769 reports were examined for eligibility after removing 14 duplicate records. Thirteen studies were qualified for review from screening the search engines records, and additional 4 studies were identified from other methods including citation search and personal contact (Fig. 1). Hence, a total of 17 studies were qualified for review investigated mastitis buffaloes in 6 Iraqi governorates including Baghdad, Nineveh, Babylon, Al-Najaf, Missan, and Al-Basrah conducted between 1982 and

2022. The qualified studies represented 9 studies reported the proportion of buffaloes affected with mastitis (Table 1), and 13 studies reported the proportion of specific pathogen(s) isolated from mastitis buffaloes (Table 2). The percentages of risk of bias due to the threat of the external and internal validity as well as the overall risk of bias were different among the studies, with a substantial threat of the external validity (Table 3).

Meta-analysis

The overall prevalence of mastitis in buffalo in Iraq was estimated at 24.61% with evidence of SCM higher than CM; 29.62% and 20.89%, respectively (Fig. 2). In this analysis, the number of investigated buffaloes was 567 from 5 studies taking into consideration that 2 studies were investigated both CM and SCM (Table 1, Fig. 2). Substantial heterogeneity was observed at overall and subgroup levels (Q-statistic $P < 0.05$, $I2 = 88.16\%$ and 85.13%, respectively; Figure 2) although heterogeneity statistics for subclinical mastitis, as a subgroup, were not estimated because it included only three studies. In addition, no evidence of heterogeneity between groups was existed ($P = 0.33$, Fig. 2). Egger regression revealed no evidence of small-study effects (bias coefficient = 0.17 with a standard error of 0.18 and a P-value of 0.39). Additionally, we run Egger regression for subgroup separately and no evidence of small-study effects has revealed; P-value = 0.62 and 0.67 for CM and SCM subgroups, respectively.

The analysis, on the other hand, indicated that the most prevalent pathogen isolated from mastitis buffaloes was *Staphylococcus* spp. (46.75%) followed by *Streptococcus* spp. and other miscellaneous bacteria at 32.96%, 19.04%, respectively (Fig. 3). In this analysis, number of samples applied for bacteriological isolation was 1,442 from 10 studies taking into consideration that 5 studies isolated different pathogens, 4 studies targeted isolation of *Staphylococcus* spp., one study targeted *Listeria*

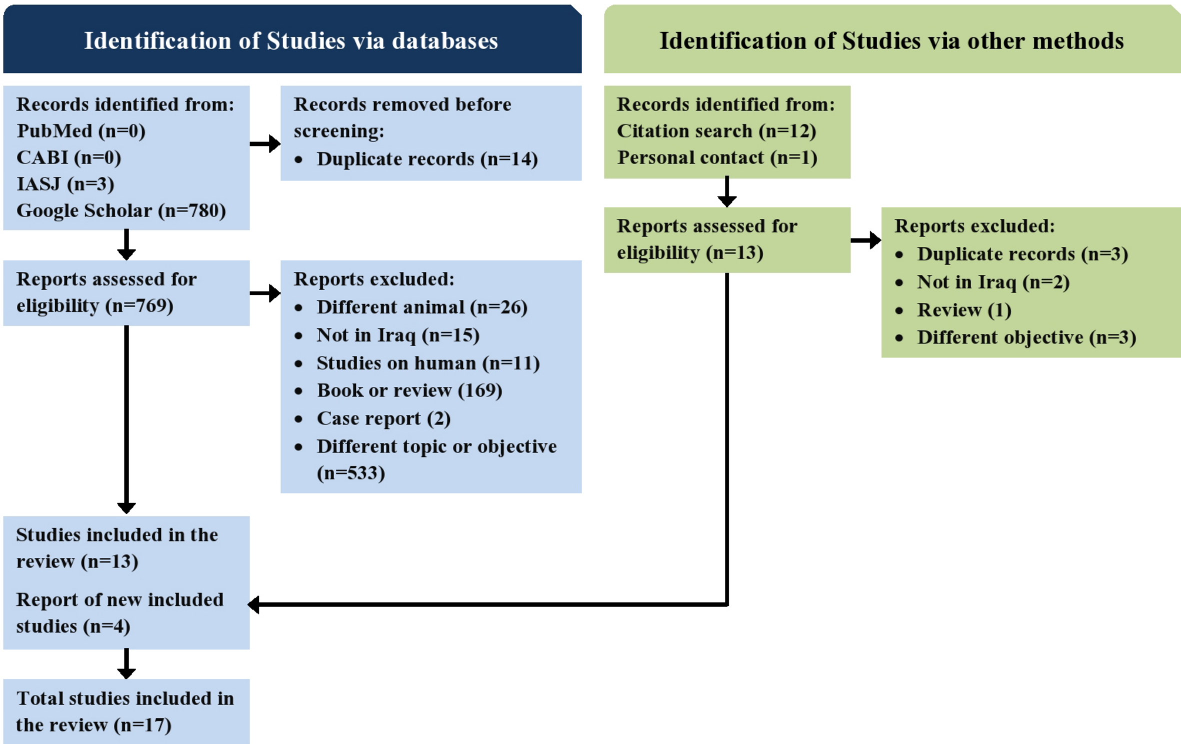

Fig. 1. Flow diagram of article selection process applied in the systematic review for the evidence of mastitis in buffalo in Iraq. Keywords (buffalo, mastitis, Iraq) were used on August 24, 2021.

Table 1. Proportion of mastitis cases in lactating buffaloes in Iraq.

Author	Year	Province	N	Mastitis Animals	Diagnosis	Type of mastitis
Abdul-Razak	1982	Baghdad Missan	151 144 [a]	38 46	Clinical Culture	CM SCM
Al-Kass	2005	Nineveh	148 [b]	20 24	Clinical Whiteside test	CM SCM
AL-Saadi and ALShakh	2015	Babylon	80	29	Clinical	CM
Al-Ziany and Al-Jeburii	2015	Baghdad	83	10	Clinical	CM
Abdul-Razaaq and Dere [c]	2018	Al-Basrah	NM	65	Clinical	CM
Al-Dujaily *et al.*	2019	Al-Najaf	103	43	CMT (+++)	SCM
Ibrahim	2019	Baghdad	80	NM [d]	-	-
Dahl *et al.* [e]	2021	Nineveh	16	1	CMT	SCM
Saadoon	2022	Nineveh	800 [f]	85 219	Clinical CMT	CM SCM

CM: clinical mastitis; SCM: subclinical mastitis; CMT: California mastitis test; NM: not mentioned.
[a] The 144 buffaloes were already included in the 151 ones investigated for CM.
[b] Although the author mentioned this number as a samples, he considered it as a number of animals, too.
[c] This study reported only animals affected with several diseases over a period of 7 years documented at the Veterinary Hospital.
[d] Numbers were not mentioned in this study, and we were not able to calculate it because the authors reported mastitis rate as mean ± standard error.
[e] This study included only animals examined at the Veterinary Teaching Hospital.
[f] The author indicated that this number is representing number of samples examined not number of animals investigated.

monocytogenes, one study targeted *Salmonella* spp., and one study targeted *E. coli*, (Table 2, Fig. 3). Substantial heterogeneity was observed at subgroup level for "*Staphylococcus*" and "Other Bacteria" (Q-statistic P < 0.05, I2 = 98.48% and 96.74%, respectively; Fig. 3) although no evidence of heterogeneity was observed for "*Streptococcus*" subgroup (Q-statistic P = 0.13, I2 = 46.48%). In addition, evidence heterogeneity between groups was existed (P = 0.04, Fig. 3). Finally, Egger regression for subgroups revealed no evidence of small-study effects for "*Streptococcus*" subgroup (bias coefficient = 0.31 with a standard error of 0.16 and a P-value of 0.11) and "Other Bacteria" subgroup (bias coefficient = 0.28 with a standard error of 0.14 and a P-value of 0.11), whereas "*Streptococcus*" subgroup showed evidence of small-study effects; bias coefficient = 0.37 with a standard error of 0.08 and a P-value of 0.04.

Discussion

The study conducted here is the first local investigation examined and quantified the evidence and identified knowledge gaps in studies investigated mastitis in buffaloes in Iraq. The investigation identified 17 studies used buffaloes in 6 Iraqi governorates. The analysis revealed that the prevalence of CM and SCM was 20.89% and 29.62%, respectively, and the most prevalent pathogen in mastitis buffaloes was *Staphylococcus* spp. followed by *Streptococcus* spp.

The systematic review conducted here revealed that the majority of the studies were performed in 2010 and later years except for two studies in 1982 and 2005. It is not clear why studying mastitis in buffaloes in Iraq started late, although the country reported 141,000, 117,778, and 283,839 heads of buffalo in 1986, 2001, and 2008, respectively, except Kurdistan region (Agricultural Statistical Atlas, 2011), and their milk has been being used as an important source for local dairy products. Performing such studies could improve udder health and consequently the production and the profitability of the local buffalo industry. In addition, buffaloes in only 6 governorates have been investigated; nevertheless, buffaloes are found in all Iraqi governorates. Therefore, further studies in other governorates are required to better understand the epidemiology of mastitis in buffaloes in Iraq.

Majority of retrieved studies have focused on the identification of causative pathogens isolated from mastitis buffaloes, particularly *Staphylococcus* spp. Some studies reported the proportions of CM and/or SCM episodes. However, factors affecting incidence of mastitis in buffaloes in Iraq are still not adequately investigated. The only conclusive result was reported by Abdul-Razak (1982) which indicated that mastitis is increased the age of the animal, and animals in first two months of lactation are at greater risk of mastitis. Moreover, Abdul-Razaaq and Dere (2018) found that winter is a risk factor for mastitis due to increase of the humidity. Results of other studies are considered inconclusive. For instance, Saadoon (2022) investigated animals' age, stage of lactation, herd size, type of feeding, and season as potential risk factors for mastitis; however, the unit of that study was sample rather than animal. Therefore, possible overestimate or underestimate of the effect existed due to including more than one sample for a single animal in the analysis.

Risk of bias in identified studies was substantial, particularly those for external validity. That is, all studies overlooked calculating sample size and identifying inclusion and exclusion criteria. Sample size determination is crucial in observational studies in order to produce a precise estimate (Vandenbroucke *et al*., 2014; Munn *et al*., 2015), and identification of inclusion and exclusion criteria is an important strategy to ensure including only eligible animals in the study to avoid over or underestimating

Table 2. Studies investigated presence of bacteria in milk sampled from buffaloes with different status of mastitis at various provinces in Iraq.

Author	Year	Province	N	Mastitis Status	Isolates	Isolated Bacteria	Method of detection
Abdul-Razak	1982	Baghdad, Missan	73	CM	68	Different types [1]	
			531	SCM	66	Different types [1]	
Al-Kass	2005	Nineveh	41	CM, SCM	44	Different types [2]	Different media
Sheet	2010	Nineveh	100	NM	78	*S. aureus*	Mannitol salt
Abbas and Jaber	2012	Al-Basrah	100	NM	3	*Listeria monocytogenes*	Tryptose agar
Khudaier *et al*.	2014	Al-Basrah	215	Clinically healthy	22	*S. aureus*	Mannitol salt
Yousif and Al-Hashimi	2014	3 different provinces: middle of Iraq	150	Clinically not mastitis buffaloes	0	*Salmonella* spp.	Different cultures
Al-Ziany and Al-Jeburii	2015	Baghdad	332	N=14 CM	15	Different types [3]	Blood agar, Edward, Mannitol salt, MacConkey
				N= 79 SCM	75	Different types [4]	
Khaleel *et al*.	2016	Al-Basrah	180	NM	40	*S. aureus*	Mannitol salt
Abdul wahid *et al*.	2017	Al-Najaf	100	N=12 CM	36	*E. coli*	Blood agar
				N=24 clinically healthy	15	*E. coli*	MacConkey
					10	*E. coli*	EMB
Khaleel *et al*.	2018	Al-Basrah	17	NA [5]	5	*S. aureus*	Molecular diagnosis
Al-Dujaily *et al*.	2019	Al-Najaf	172	SCM (CMT +++)	124 [6]	*S. aureus*	Blood agar
Lafta	2020	Al-Basrah	15	CM	NM	Proteus mirabilis, and Enterobacter cloacae	Nutrient broth, Blood agar, MacConkey
Saadoon	2022	Nineveh	85	CM	85	Different types [7]	Blood agar, Nutrient agar, MacConkey
			219	SCM	219	Different types [6]	

Abbreviations: CM: clinical mastitis; SCM: subclinical mastitis; NA: not applicable; NM:not mentioned.
[1] Included different isolates of: *S. aureus*, *Staph. epidermidis*, *Strept. agalactiae*, *Strept. dysgalactiae*, *Corynebacterium pyogenes*, and other *Streptococci*.
[2] Included different isolates of: *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium bovis*, and atypical *Mycobacterium*. Additionally, Gram-negative bacilli were identified.
[3] Included different isolates of *Strept. uberis*, *Strept. agalactiae*, *Strept. dysgalactiae*, *Strept. equi*, *Strept. canis*, *Staph. sciuri*, and *Lactococcus garvieae*.
[4] Included different isolates of: *Strept. uberis*, *Strept. agalactiae*, *Strept. thoraltensis*, *Strept epidermidis*, *Staph. chromogens*, *S. aureus*, *Staph. sciuri*, *Staph. lentus*, *Staph. xylosus*, *Staph. haemolyticus*, *Staph. equi*, *Enterococcus casseliflavus*, *Enterococcus gallinarum*, *Enterococcus faecium*, *Lactococcus garvieae*, *Klebsiella rosea*, and a bacterium the authors named it as *A. otitis*.
[5] This study is a follow-up for a previous study by Khaleel *et al*. (2016) to identify the 23S rRNA gene in the previous isolates.
[6] The authors did not mention the number of the isolates; however, they reported that *S. aureus* were isolated from 72% of milk samples.
[7] Included different numbers of: *S. aureus*, *Strept. agalactiae*, *Strept. pyogenes*, *Corynebacterium bovis*, *E. coli*, *Pseudomonas aeruginosa*, and *Pasteurella multocida*.
[8] Included different numbers of: *Staph. chromogens*, Staph. xylosus, *Strept. agalactiae*, *Strept. dysgalactiae*, *Strept. uberis*, *Proteus vulgaris*, *Klebsiella pneumonae*, *E. coli*, *Corynebacterium bovis* and *Pasteurella multocida*.

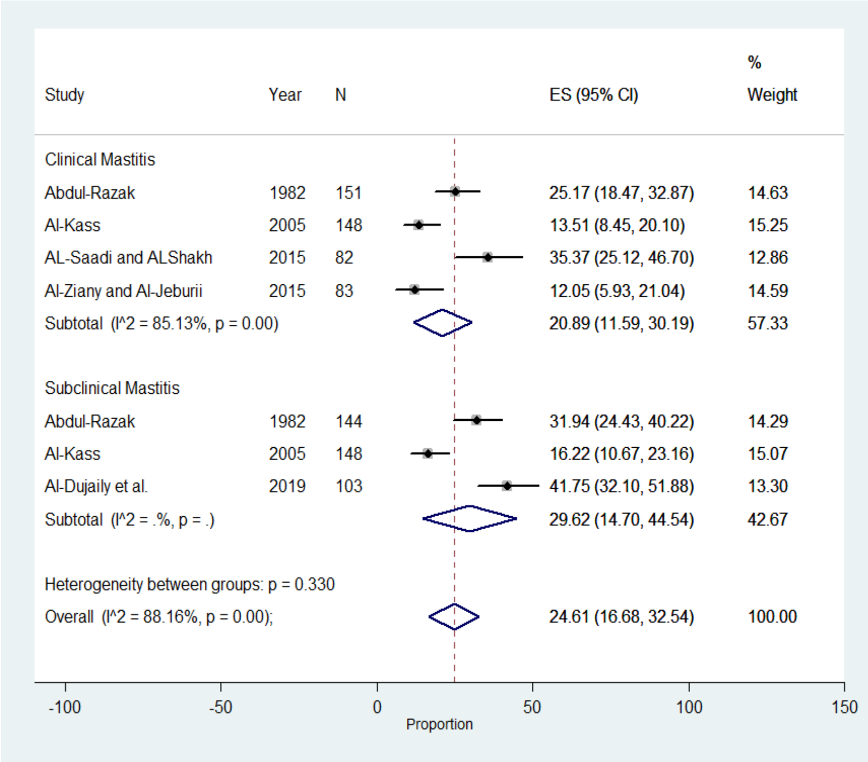

Fig. 2. Forest plot for studies reported mastitis (CM and/or SCM) in Iraq. Each study was identified by the last name of the first author and the year of publication. The squares indicated the size of individual study's effect as a proportion with corresponding confidence interval (the horizontal lines). The diamonds show the effect size estimate for subgroups and overall, as well. The width of each diamond shows the confidence interval of that estimate.

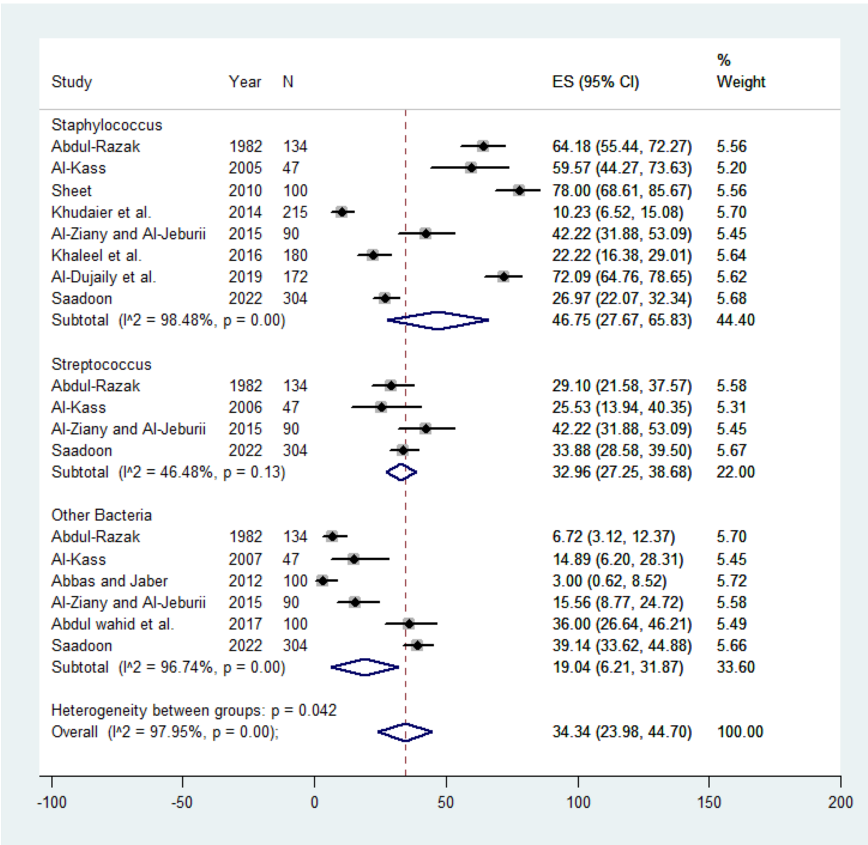

Fig. 3. Forest plot for studies reported the pathogens isolated from mastitis buffaloes in Iraq. Each study was identified by the last name of the first author and the year of publication. The squares indicated the size of individual study's effect as a proportion with corresponding confidence interval (the horizontal lines). The diamonds show the effect size estimate for subgroups and overall, as well. The width of each diamond shows the confidence interval of that estimate.

the study effect. In addition, majority of the studies did not adequately explain the method they used for random selection of study animals. Random selection is important strategy to increase the generalization of the study result and to make the sample close to represent the target population (Munn *et al*., 2015). Moreover, most of the studies did not describe the animals included in the study in details. On the other hand, the internal validity criteria were taken into consideration better than that of external validity. That is, most identified studies paid attention to the standard criteria used to measure the outcome of interest, although they did not clearly define the outcome of interest. Finally, most studies appropriately analyzed the data. Therefore, the percent of risk of bias due to threatening the internal validity was lower than that of external validity.

Table 3. Criteria used to assess (with percentage of) risk of bias in studied qualified for review to study evidence of mastitis in buffaloes in Iraq*

Study	Criteria for assessment of risk of bias								Risk of bias percentage (%)		
	External Validity				Internal Validity				External validity	Internal validity	overall
	1	2	3	4	5	6	7	8			
Abdul-Razak (1982)	100	100	75	100	50	50	0	0	93.8	25	59.4
Al-Kass (2005)	100	100	0	50	100	75	0	0	62.5	43.8	53.1
Sheet (2010)	100	100	75	50	100	0	0	0	81.3	25	53.1
Abbas and Jaber (2012)	100	100	75	50	100	75	0	0	81.3	43.8	62.5
Khudaier *et al*. (2014)	100	100	0	50	100	75	0	0	62.5	43.8	53.1
Yousif and Al-Hashimi (2014)	100	100	75	75	50	0	0	75	87.5	31.3	59.4
AL-Saadi and ALShakh (2015)	100	100	75	100	75	100	75	50	93.8	68.8	81.3
Al-Ziany and Al-Jeburii (2015)	100	100	75	100	75	50	50	50	93.8	56.3	75
Khaleel *et al*. (2016)	100	100	75	0	100	75	0	0	68.8	43.8	56.3
Abdul wahid *et al*. (2017)	100	100	75	75	100	75	75	50	87.5	75	81.3
Al-Dujaily *et al*. (2019)	100	100	75	75	100	75	50	75	87.5	75	81.3
Ibrahim (2019)	100	100	75	100	100	75	75	75	93.8	81.3	87.5
Lafta (2020)	100	100	75	100	50	75	50	75	93.8	62.5	78.1
Saadoon (2022)	100	100	75	0	100	0	0	0	68.8	25	46.9

* Three studies included those by Abdul-Razaaq and Dere (2017), Khaleel *et al*. (2018), and Dahl *et al*. (2021) were not subjected to the assessment and estimation of the percentage of risk of bias because they included only number of buffaloes affected with mastitis examined at veterinary facility [those by Abdul-Razaaq and Dere (2017), and Dahl *et al*. (2021)] or different objective [Khaleel *et al*. (2018)].

The data analyzed in the meta-analysis here indicated that the prevalence of SCM higher than CM; 29.62% and 20.89%, respectively. This result is in line with the study of Krishnamoorthy *et al.* (2021) who indicated that prevalence of SCM is higher than CM in buffalo globally. A potential explanation for this pattern of episodes is that the susceptibility of buffaloes to mastitis is low as a function of the anatomical characteristics of the udder and the alkaline property of the milk in buffalo (Purohit *et al.*, 2014; Briñez *et al.*, 2000). Consequently, the infection would not be developed to a clinical form and detected as SCM. On the other hand, the prevalence of SCM reported here is considered lower than that reported in the countries with the largest number of buffaloes such as India 66.33% (Kashyap *et al.*, 2019) and 35.2% (Rahman *et al.*, 2019), Pakistan 38.8% (Hussain *et al.*, 2018) and 66% (Ali *et al.*, 2021), and Egypt 44.3% (Ahmed *et al.*, 2018). The prevalence of CM in our analysis was greater than that estimated in other countries such as India 11% (Kashyap *et al.*, 2019) and Pakistan 10.2% (Hussain *et al.*, 2018). One possible reason for the difference in the prevalence of CM and SCM in Iraq and other countries could be attributed to the difference in the herd management and hygiene milking measures. Providing good herding with clean environment and hygiene milking procedures have essential role in decrease of mastitis incidence, although it would not completely eliminate it (Zigo *et al.*, 2021). Another reason is that local studies identified in the current review included high risk of bias which could lead to over or underestimate of the prevalence. Therefore, more local studies that follow the standard epidemiological tools are required to confirm the prevalence of mastitis in buffalo in Iraq.

The analysis conducted here revealed a substantial heterogeneity in the studies reported prevalence of mastitis. However, Egger regression showed no evidence of small-study effects. The heterogeneity revealed here is most likely statistical as the results of identified studies were not consistent (Fletcher, 2007) because some studies reported high prevalence compared to other studies in the same analysis. Moreover, no effect of small-studies was existed because both small and large studies reported high or low prevalence. For instance, the study by Al-Saadi and AlShakh (2015) indicated that prevalence of CM using 82 buffaloes was 35%, whereas Al-Ziany and Al-Jeburri (2015) reported 12% CM using 83 buffaloes. Furthermore, by Al-Dujaily *et al.* (2019) identified 41.75% of 103 buffaloes as SCM, whilst Al-Kass (2005) revealed 16% of 148 of buffaloes had SCM. Therefore, heterogeneity with no small study effect has been observed in our analysis. A possible cause of heterogeneity is that the studies were conducted in different governorates; thus, the differences in the prevalence could have been true.

The data showed that *Staphylococcus* spp. was mostly isolated in mastitis buffaloes, followed by *Streptococcus* spp. and other miscellaneous bacteria. Our results reported here are in line with several studies worldwide. For instance, *Staphylococcus* spp. was reported as a most prevalent pathogen isolated from mastitis buffaloes in India (Sentitula *et al.*, 2012), Pakistan (Ali *et al.*, 2021), Egypt (Zaitoun, 2008), Brazil (Pizauro *et al.*, 2014), and Venezuela (Briñez *et al.*, 2000). In addition, *Streptococcus* aureus has been reported as the greatest important pathogen that can cause mastitis in dairy Mediterranean Buffaloes (Guccione and Ciaramella, 2017), and frequently isolated in Egypt (El-Ashker *et al.*, 2015). In contrast, *Streptococcus* spp. also represented a considerable pathogen isolated from mastitis buffaloes. Our result agrees with the studies of Zaitoun (2008) and Pizauro *et al.* (2014) that *Streptococcus* spp. was the second predominant pathogen identified in mastitis buffaloes. Finally, isolation of several other pathogens reflects the multi-pathogenic nature of mastitis (Purohit *et al.*, 2014).

In the current analysis, a substantial heterogeneity was observed in the studies isolated different pathogens from mastitis buffaloes, except that for the subgroup of "*Streptococcus*". Again, the heterogeneity observed in this analysis is most likely statistical because there was inconsistency in the percentage of pathogens isolated from mastitis buffaloes included in the analysis, except that of "*Streptococcus*" subgroup (Fletcher, 2007). In contrast, Egger regression revealed no evidence of small-study effects except that for "*Streptococcus*" subgroup. That is, both small and large studies reported high or low percentages of *Staphylococcus* spp. and other pathogens, whereas only the smallest study reported the smallest percentage of *Streptococcus* spp. indicating the effect of small-study (Fig. 3). More studies are required to produce more generalized evidence.

Study Limitations

The analysis performed here included some limitations. First, the identified studies were conducted in 6 Iraqi governorates which limit our knowledge about mastitis in buffaloes countrywide as buffalo is found in almost all of the 18 Iraqi governorates except Duhok and Sulaymaniyah (Agricultural Statistical Atlas, 2011). Second, we were not able to quantify risk factors associated with mastitis in buffalo because very few studies (one or two) examined some pertinent factors. Finally, risk of bias in identified studies was high; however, this was because of the external validity threatening rather than internal validity. Thus, the results of a single study were reliable although it is difficult to be generalized.

Conclusion

The study conducted here concluded that mastitis is evident in buffalo in Iraq, with evidence that SCM is more prevalent than CM, and *Staphylococcus* spp. is a predominant pathogen that can be isolated from mastitis buffalo followed by *Streptococcus* spp. However, several knowledge gaps have been realized. First, no data is available about mastitis in buffalo in several Iraqi governorates. Second, incidence of mastitis during first month of lactation as a most risk period of mastitis is not known. Third, risk factors associated with mastitis are not clearly and adequately examined. Such factors include, but not limited to, parity, season, milk yield, stage of lactation, and exposure to other diseases such as metritis, ketosis, and lameness. Fourth, economic burden due to mastitis is not yet studied. Finally, it is important to apply standard epidemiological tools in designing local studies that aim to estimate incidence/prevalence and identify risk factors of mastitis in buffalo.

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Conflict of interest

The authors declare that there is no conflict of interest related.

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