



## Epidemiological investigation of mastitis in lactating buffalo cows in Nineveh governorate, Iraq

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### Abstract

The study's objective was to investigate the incidence and predisposing factors of mastitis in lactating buffalo cows in the Nineveh governorate. A total of 160 recently calved buffalo cows were followed up in a prospective study during the first three months of lactation. Cases of clinical mastitis (CM) were investigated, and milk was sampled to identify the increase in somatic cell count (SCC) as an indicator of subclinical mastitis (SCM). Several epidemiological factors that can predispose to mastitis were also investigated. The incidence of CM was 9.4%, mostly recorded during the first ten days (4 days on average). The average CM duration per case was  $35 \pm 4.9$  days. Different changes and clinical findings were revealed in milk and affected udder. The incidence of SCM (i.e.,  $SCC > 200,000$  per mL) was 83.75%, mostly recorded during the first month of lactation. Furthermore, the odds of SCM were high in winter. The study concluded that the incidence of CM in buffalo cows is very low. The chronic form characterizes CM, SCM is more common than CM, and winter is a predisposing factor for the infection. Examination of several control measures of mastitis is important to improve the production and hygiene of buffalo milk in Nineveh governorate.

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### Introduction

Mastitis is an inflammation of the mammary gland characterized by physical and/or chemical and pathological changes in milk accompanied or not by systemic reactions (1). Although multi-pathogens can cause mastitis, bacteria are considered the most common causative agents (2). Risk of mastitis is increased during the periparturient period, with the greatest incidence rate during the first month of the lactation period (3). However, the susceptibility of buffaloes to mastitis is considered lower than that of cows (4). This is attributed to the anatomical characteristics of the udder in buffaloes that minimize the opportunities of microorganisms' invasion (2). Such characteristics include long and narrow teat canal, thick keratinized epithelial layer of the streak canal, and tightly closed teat orifice (5). In addition, the buffalo milk is more alkaline compared to cow milk, which can also contribute to decreasing the risk of

mastitis in buffaloes (6). Buffalo is considered the second most essential species for dairy production after cow, and the price of buffalo milk can reach twice that of cow (7,8). Mastitis is one the costliest disease faces dairy producers (9). In Iraq, buffalo is mainly raised for production of milk, yogurt, cream, and ice-cream production. Up to our knowledge, only three studies have investigated mastitis in buffalo in Nineveh governorate, including those of Al-Kass (10), Sheet (11), and Saadoon (12). However, those studies have focused on isolation of the bacteria that potentially caused the cases. In addition, the majority of the studies were based on milk samples rather than animals as a unit of the study.

Therefore, several knowledge gaps exist concerning epidemiology of mastitis in lactating buffalo cows in Nineveh. Such gaps include, but not limited, incidence of clinical and subclinical mastitis (CM and SCM) during early lactation, duration of mastitis, and predisposing factors of the

infection. Therefore, this study was conducted to investigate incidence and predisposing factors of mastitis in lactating buffalo cows in Nineveh governorate.

## **Materials and methods**

### **Ethical approval**

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the College of Veterinary Medicine, University of Mosul, with ethical approval number UM.VET.2021.057.

### **Study animals**

Recently calved buffalo cows raised by private owners around Tigris River across Nineveh governorate, Iraq were considered for inclusion in this study. To minimize the loss of follow-up and because not all local buffalo owners permit investigating their animals in repeated visits, included animals were only from owners who expressed the willingness to participate and agreed that their animals are examined in the study. A sample size of 160 animals was sufficient to achieve a precision between 4-5% and a confidence of 95% (13), based on the assumption that the expected mastitis rate in female buffaloes is 10% (12) and the number of buffaloes in Nineveh Governorate is 17,750 heads according to the Nineveh Governorate Agriculture Directorate. Sample size calculation was conducted using the OpenEpi website [\[available at\]](#).

### **Study design**

Study buffalo cows calved between October 1<sup>st</sup>, 2021 and May 1<sup>st</sup>, 2022 were included in a prospective investigation. Study animals were followed up during the first three months of their lactation to record and follow up the incidence of CM. In addition, milk samples were collected from each animal at 15 days intervals during the entire investigation period to examine the incidence of increase in SCC as an indicator of SCM.

### **Definition of mastitis**

In this study, buffalo cows were considered affected with CM when they produced milk with changes in the physical characteristics such as texture, color, or contained flakes or clots, accompanied or not accompanied by a clinical finding on the udder such as increased size, redness or pain (1). On the other hand, buffalo cows were considered affected with SCM when they produced milk without apparent physical changes in milk or did not suffer from an apparent clinical finding on the udder, but they had SCC more than 200,000 cells per mL of milk (14,15).

### **Somatic cell count**

In this study, direct microscopic somatic cell count was used to determine the SCC in the collected milk as previously described by Fitts *et al.* (16) and Coles (17). In

brief, milk films were prepared to ensure that 0.01 mL of milk was spread on an area of 1 cm<sup>2</sup> and stained using the Newman-Lampert stain. Somatic cells were counted in 30 fields around the film using the oil-immersion objective. The average number of cells counted in 30 fields was multiplied by 392,974.2, which constituted the microscopic factor.

### **Data collection**

The following data were collected for each study animal: calving date, lactation number (1<sup>st</sup> lactation, 2<sup>nd</sup> or later lactations), dystocia (yes, no), retained fetal membranes (yes, no), metritis (yes, no), lameness (yes, no), CM (yes, no), date of CM incidence, duration of CM in days, clinical findings on milk and udder, and SCM ( $\leq 200,000$  SCC/mL milk,  $> 200,000$  SCC/mL milk). In addition, area where buffaloes were located was classified as north or south of the governorate; the center of Mosul city was considered the cut-point between north and south of the entire governorate. Finally, season of calving was classified as autumn (October and November), winter (December, January, February), and spring (March, April, and May). Months from June to the end of September (i.e., summer season) were not included because the investigation was conducted during limited time.

### **Statistical analysis**

The incidence of mastitis constituted the proportion of buffaloes that experienced new cases of CM or SCM, as previously defined, during the first three months of lactation (18). The Odds Ratio (OR) was used as an epidemiological criterion to measure the association between the incidence of CM or SCM as a function of investigated exposure factors (19). One-way ANOVA and Duncan's test were used to examine the evidence of a difference and multiple pairwise comparisons between SCC means across sampling periods, respectively (20). In all analyses, a *P*-value of  $\leq 0.05$  two-tailed was considered significant. The statistical analyses were performed using STATA 13.0; StataCorp, College Station, TX, USA.

## **Results**

### **Clinical mastitis**

The overall incidence of CM was 9.4%, mostly recorded during the first ten days (4 days on average) except for one case recorded on day 34 of lactation (Figure 1). The cases of CM were characterized by the chronic form, with an average duration of  $35 \pm 4.9$  days (Figure 2). Some of CM cases 46.6% turned to SCM characterized by a high SCC (mean = 882,199; min = 301,280; max = 3,274,784), whereas other cases were cured 20%. Buffaloes affected with CM observed different clinical findings on milk and udder without systemic reactions (Table 1, Figure 3). Furthermore, the statistical analysis revealed that the epidemiological factors did not change the odds of incidence of CM, except that there was a tendency (*P* = 0.08) that the odds of CM increased in winter (Table 2).

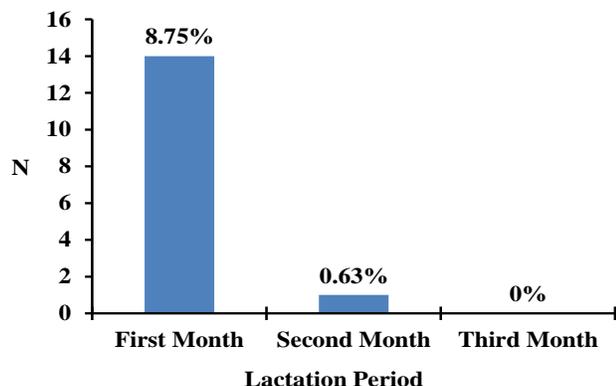


Figure 1: Incidence of clinical mastitis during the first three months of lactation in lactating buffaloes in Nineveh governorate, Iraq (The overall incidence was 9.4%).

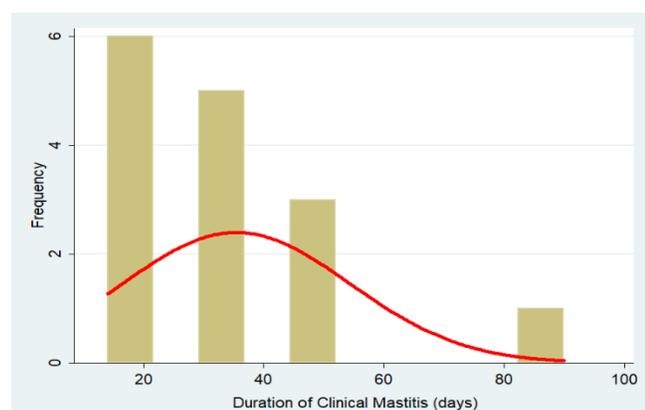


Figure 2: Distribution of clinical mastitis duration in lactating buffaloes (red line), with a minimum value of 14 days, a maximum of 90 days, and an average of 35 days.

Table 1: Clinical findings observed on milk and udder of lactating buffaloes affected with CM during the first three months of lactation

Clinical Finding	Frequency	Percentage
Changes in milk		
Changes in the color	13	86.6%
Changes in the texture	3	20%
Presence of flakes or clots	6	40%
Enlargement on the udder	3	20%
Affected quarter		
Right hind	5	33.3%
Right front	4	26.6%
Left hind	2	13.3%
Left front	1	6.6%
Whole udder	1	6.6%

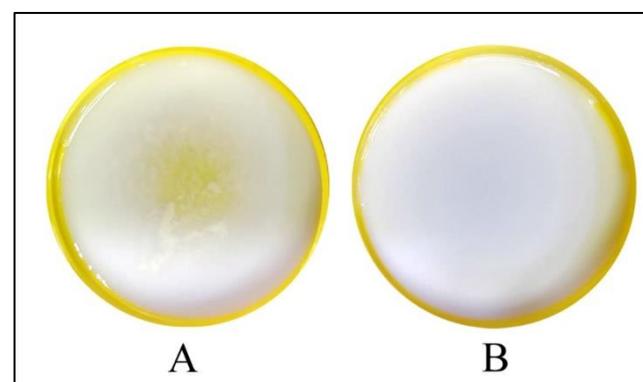


Figure 3: Milk sample from affected udder showed presence of clots (A) compared to that of normal udder (B).

Table 2: Odds of clinical mastitis in lactating buffaloes during first three months of lactation as a function of different exposure variables

Variables	Clinical Mastitis		Odds Ratio	95% CI	P	
	Yes (15)	No (145)				
Area	North	6	61	1.00	Referent	-
	South	9	48	1.09	0.37, 3.22	0.88
Lactation	First	6	44	1.53	0.51, 4.56	0.45
	Second or later	9	101	1.00	Referent	-
Season	Autumn	3	29	1.78	0.38, 8.48	0.47
	Winter	8	45	3.07	0.87, 10.79	0.08
	Spring	4	69	1.00	Referent	-
Dystocia	Yes	2	0	-	-	-
	No	13	145	-	-	-
Retained fetal membranes	Yes	1	0	-	-	-
	No	14	145	-	-	-
Metritis	Yes	0	7	-	-	-
	No	15	138	-	-	-

The incidence of SCM (SCC > 200,000 cells / mL; Figure 4) was 83.75%, mostly recorded during the first month of lactation, with an average of 18 days of lactation. The incident of SCM gradually decreased as the lactation proceeded (Figure 5). This incidence did not include the SCM that turned from CM. The number of somatic cells in buffaloes with SCM was the greatest during the first 15 days of lactation and then decreased as the lactation proceeded (Table 3).

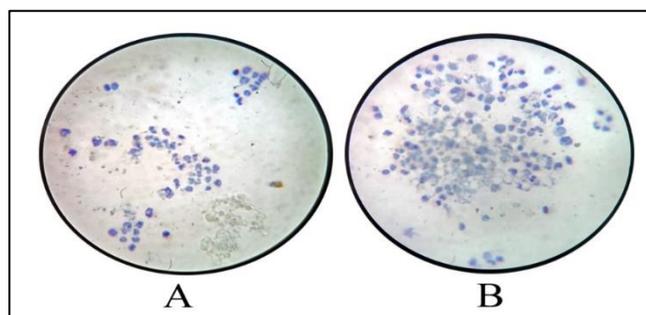


Figure 4: Somatic cell counts from buffalo milk stained with the Newman-Lampert stain. (A) showed a total of 42 cells per a field, and (B) showed a total of 110 cells per a field.

Moreover, the statistical analysis revealed that the epidemiological factors did not change the odds of incidence of CM, except that the odds of CM were 4.5 times greater in winter compared to autumn (Table 4).

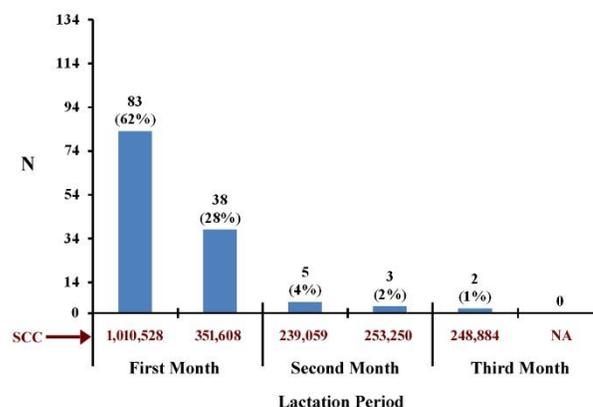


Figure 5: Incidence of subclinical mastitis during the first three months of lactation in lactating buffaloes in Nineveh governorate, Iraq (Numbers in red color indicate means of SCC).

Table 3: Means of SCC per mL milk in lactating buffaloes affected with SCM during the first three months of lactation

Month	Sample	N	SCC mean	95% CI		Significance Level
				Lower	Upper	
First	1	83	1,010,528	710,698	1,310,357	a
	2	109	652,073	500,866	803,280	b
Second	3	68	481,586	407,167	556,005	b c
	4	67	442,438	393,521	491,355	b c
Third	5	59	408,072	367,183	448,960	b c
	6	70	381,934	354,148	409,719	c

Different letters indicated significant differences at  $P \leq 0.05$ .

Table 4: Odds of subclinical mastitis in lactating buffaloes (SCC > 200,000 cells / mL) during the first three months of lactation as a function of different exposure variables

Variables	Subclinical mastitis		Odds Ratio	95% CI	P	
	Yes (83)	No (69)				
Area	North	33	25	1.00	Referent	-
	South	50	34	1.11	0.56, 2.19	0.76
Lactation	First	27	16	1.29	0.62, 2.70	0.49
	Second or later	56	43	1.00	Referent	-
Season	Autumn	11	18	1.00	Referent	-
	Winter	33	12	4.50	1.66, 12.23	0.003
	Spring	37	29	2.09	0.85, 5.10	0.11
Dystocia	Yes	0	0	-	-	-
	No	83	59	-	-	-
Retained fetal membranes	Yes	0	0	-	-	-
	No	83	59	-	-	-
Metritis	Yes	6	1	4.52	0.53, 38.58	0.17
	No	77	58	1.00	Referent	-

## Discussion

The study conducted here was the first prospective study in Nineveh governorate investigated the incidence of mastitis in lactating buffalo cows during the first three months of lactation. It is also the first study in Nineveh indicated that the CM in buffalo is characterized by the chronic form. Our study confirmed that SCM is more common than CM in buffalo, and is characterized by high SCC that persists for a long time before it returns to normal values.

The results of this study revealed that the incidence of CM was 9.4%. This percentage is lower than the studies of Al-Kass (10) in Nineveh and Al-Zainy and Al-Jeburii (21) in Baghdad that reported 13.5 and 12.05%, respectively. One potential reason is that the previous studies targeted buffalo herds affected with CM to isolate the bacteria that caused the infection, which may overestimate the incidence of CM. Another possible explanation is that our study targeted only the first three months of lactation; however, the risk of infection is the greatest during this period, particularly the first month (3) which is also revealed in our study. Results of Sheet (11) and Saadoon (12) studies in Nineveh are not comparable to our results as they were sample-based rather than animal-based.

The study conducted here indicated that the incidence of CM and SCM was higher during the first month of lactation. This result is in line with Waller (3) who stated that the risk of mastitis is considered the greatest during early lactation. The risk of infection is increased during these days due to the stress caused by calving, the negative energy balance during this period, and the start of lactation, which can lead to an increase of corticosteroids levels in the blood making the animals less resistant to infectious diseases (3,22).

Our results revealed that the CM is characterized by the chronic form; the mean duration of infection was 35 days. Explanation of this pattern of infection requires more studies. One possible reason could be a function of not using appropriate medications for the cases as mastitis is considered a multi-pathogens infection (2), or due to insufficient duration of treatment, or due to the fact the environment of the buffalo makes the animals vulnerable to pathogens, as buffalo prefers mud, which exposes the udder to various environmental pathogens for long periods, thus reducing the recovery rate and the infection continues as a chronic form. A clean, non-humid and adequately ventilated environment is fundamental in the prevention of mastitis in dairy animals (23).

The current study showed that the incidence of SCM is higher than that of CM, 83.75 and 9.4%, respectively. This result is in line with local studies such as Al-Kass (10) and Abdul-Razak (24), as well as other studies in different countries such as India (25) and Pakistan (26). An explanation of this pattern of infection is that the susceptibility of buffaloes to clinical mastitis is considered low (4), which is attributed to the anatomical characteristics

of the udder in buffaloes that minimize the opportunities of microorganisms' invasion (2), such as long and narrow teat canal, thick keratinized epithelial layer of the streak canal, and tightly closed teat orifice (5). All these factors might increase the odds of subclinical infection rather than clinical episode.

Buffaloes affected with CM in our study showed different clinical findings on milk and affected udder. These changes are the function of inflammatory reactions due to invasion of the udder by pathogenic microorganisms that lead to the secretion of cytokines and prostaglandins responsible for the clinical symptoms of mastitis (27). In addition, continuous growth of bacteria inside the udder causes damage to the mammary gland cells and the blood-milk barrier, allowing the blood and its components to mix with milk leading to changes in milk from the affected udder (28). On the other hand, epidemiological factors that can be associated with the infection did not show an effect in the increase of the odds of the infection except that the winter increased the odds of both CM and SCM, which is in line with the finding of Abdul-Razaaq and Dere (29). A possible reason is that the humidity in winter in our region is increased which increases the growth of bacteria in the environment surrounding the animals increasing the odds of the infection (30).

## Conclusion

The study concluded that the incidence of CM in buffalo cows is very low, CM is characterized by the chronic form, SCM is more common than CM, and winter is a predisposing factor for the infection. Examination of several control measures of mastitis is important to improve the production and hygiene of buffalo milk in Nineveh governorate.

## Acknowledgments

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## Conflict of interest

The authors declare that there is no conflict of interest in the research.

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## تحرّ وبائي عن التهاب الضرع في إناث الجاموس الحلوبية في محافظة نينوى

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### الخلاصة

هدفت الدراسة الحالية إلى التحري عن التهاب الضرع والعوامل المؤهبة له في الجاموس في محافظة نينوى. تمت متابعة مائة وستون أنثى من إناث الجاموس الوالدة حديثاً خلال الأشهر الثلاثة الأولى من إدرار الحليب. تمت متابعة حالات التهاب الضرع السريري، وأخذت عينات من الحليب لتحديد الزيادة في تعداد الخلايا الجسدية بوصفها مؤشراً على التهاب الضرع تحت السريري، فضلاً عن دراسة عوامل وبائية متعددة يمكن أن تؤهب لالتهاب الضرع. بينت النتائج أن معدل حدوث التهاب الضرع السريري كان 9,4%، تم تسجيلها في الأيام العشرة الأولى (بمتوسط 4 أيام)، وكان متوسط مدة الإصابة 35 يوماً. تمت ملاحظة تغيرات عديدة في الحليب وعلى الضرع المصاب. أظهرت النتائج أن معدل حدوث التهاب الضرع تحت السريري بلغ 83,7%، تم تسجيلها أغلبها في الشهر الأول من بدء إدرار الحليب. بينت النتائج أن فصل الشتاء يعد عاملاً مؤهباً لزيادة أرجحية الإصابة بالتهاب الضرع. استنتجت الدراسة أن حدوث التهاب الضرع السريري في إناث الجاموس يعد منخفضاً ويتميز بالشكل المزمن، وأن التهاب الضرع تحت السريري يعد أكثر شيوعاً منه، وأن فصل الشتاء هو عامل مؤهب للإصابة. إن إجراء دراسات تهدف إلى الحد من التهاب الضرع تعد ضرورية لتحسين إنتاج الحليب ونوعيته في الجاموس في محافظة نينوى.