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Clinical and subclinical mastitis in buffalue in Mosul area, Iraq

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Abstract

This paper aims to investigate the occurrence of clinical and sub-clinical cases of mastitis in buffaloes, and to identify the differences in the components of the mastitis milk, to facilitate the investigation of a number of epidemiological risk aspects in relation to the occurrence of mastitis and to detect the different bacterial species associated with the disease. Eight hundred buffalo milk samples were randomly extracted from the animals of different ages in Mosul city, Iraq and examined by California mastitis test (CMT) and Ultrasonic milk analyzer. According to the clinical signs and CMT, the results indicated the prevalence of the clinical and subclinical cases of the disease in buffaloes to be 10.62% and 27.37% respectively. There was a substantial rise in the population of somatic cells /ml in both clinical and subclinical cases of mastitis. The risk factors included third trimester of lactation period, old ages 10-11 years, outdoor feeding animals, > 30 buffaloes/herd, northern and southern parts of the city, Spring and Winter seasons. In clinical instances of mastitis, the occurrence of *Staphylococcus aureus* was 25.88%, *Streptococcus agalactiae* 18.82%, *Streptococcus pyogenes* 16.47%, *Corynebacterium bovis* 14.11%, *Escherichia coli* 10.58%, *Pseudomonus aeruginosa* 7.05%, and *Pasteurella multocida* 7.05%. In the subclinical cases of mastitis, the prevalence of *Staphylococcus chromogens* was 14.61%, *Staphylococcus xylosus* 12.78%, *Streptococcus agalactiae* 11.87%, Streptococcus dysagalactiae 11.41%, *Streptococcus uberis* 10.04%, *Proteus vulgaris* 10.04%, *Klebsiella pneumonae* 9.58%, *Escherichia coli* 8.21%, *Corynebacterium bovis* 7.30%, and *Pasteurella multocida* 4.10%. It is concluded that mastitis leads to a significant reduction in the levels of total solids, lactose, protein, fat, density, and a significant rise in the levels of pH values and electrical conductivity of the milk samples of clinical and subclinical cases of mastitis.

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Introduction

The Bubaline mastitis is explained as inflammation of the parenchyma of the mammary gland of buffaloes that can be of traumatic, infectious or toxic nature and it is characterized by physical, chemical and cytological changes in the composition of the milk and pathological alterations in the mammary glandular tissue (1). In addition, it leads to the existence of a number of infectious agents including bacteria, which could be hazardous to human beings. The clinical cases of the disease typically involve the incidence of swelling, edema, discomfort and heat in the mammary gland of most clinical cases of mastitis linked to multiple variations in the physical and chemical make-up of the milk like the existence of clots, pus, blood, water, flakes and shreds containing fibrin and cellular debris associated with pathological alterations in the mammary gland tissues (2). The clinical cases of mastitis occur due to infectious agents, and may be classified according to their severity in peracute, acute, subacute and chronic cases (3). The acute cases of mastitis initiate suddenly and are generally associated with systemic reactions such as inappetence,

fever, decrease in milk production, dehydration, and occurrence of milk changes (4). The subacute cases of the disease are characteristically without clinical symptoms except a limited number of mild indications of milk alterations without systemic reactions (5), while in chronic cases of mastitis there is gradual fibrosis leading to increase in the size of the infected quarter and asymmetry of the quarters (6). The subclinical cases of mastitis cannot be detected by manual palpation or by visually examining the milk with the use of a strip cup, but detection is possible using laboratory testing (7). Generally, the cases of mastitis are caused by interaction between different microbial infections and host responses in the mammary gland, which is affected by the way the buffaloes are managed. The risk factors of mastitis include poor milking techniques, inappropriate husbandry, unsatisfactory ventilation, low hygiene standards, overcrowding and milking machine malfunction (6). The mastitis-causing bacteria are classified into contagious and environmental pathogens (1). Most infections are the result of the first type of pathogens, including *Streptococcus agalactia*, *Staphylococcus aureus*, *Mycoplasma spp*., *Pseudomonus aeruginosa*, *Arcanobacter pyogenes*, while the environmentally-related pathogens comprise *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Enterobacteriacae spp*., *Escherichia coli*, *moulds*, *yeasts* and *coagulase negative Staphylococci* (8-12). The second type of pathogens can be found in soil, faeces, litter, and on milking equipment, which are responsible for causing teat lesions that can encourage the invasion and penetration of different types of microorganisms into the udder tissues resulting in mastitis (13). The occurrence of subclinical mastitis in buffaloes exceeds than of clinical mastitis, at 18.5% compared to 9% respectively (12).

Diagnosing the disease depends on the clinical symptoms for the clinical cases of mastitis, as well as laboratory tests for the subclinical cases of mastitis. The laboratory tests include CMT, Modified Whiteside test (MWT), and the determination of somatic cell count (SCC), which is carried out microscopically or by an automatic counter (optofluorimetic method), and determination of the electrical conductivity (EC) of the milk (14,15). The healthy quarters produce milk with < 100,000 somatic cells/ml of milk and without bacterial infections, while the quarters that produce milk with > 200,000 somatic cells/ml are considered as subclinical infection (16-18). The increase of the SCC is at a gradual rate after the first 90 days of lactation and after the second or third parturitions in buffaloes (14,19).The aims of this particular research were to determine the occurrence of the clinical and subclinical cases of the disease in buffaloes in Mosul city, and also to determine the SCCs in the milk samples, to find out the differences in the components of the mastitis milk, to examine a number of epidemiological risk factors that accompany the prevalence of mastitis and to affirm the different bacterial spp.

Materials and methods

Animals and sample collection

For this research, 800 samples of buffalo milk were obtained at random from buffaloes located in the eastern, northern, western, southern and central parts of Mosul city/Iraq. The ages of the buffaloes were between 4 and 12 years. A 20 ml of milk sample was obtained from individual teats after washing and disinfecting the teats with tincture of Iodine 0.1% in sterile vials, then transported in cold boxes to the laboratory. The respiratory rate/min, heart rate/min, rumenal movements/5min and rectal temperature of the animals were recorded. The mammary glands of the buffaloes were examined through manual palpation to detect clinical cases of mastitis. The milk samples were physically examined and also visually inspected to detect abnormal changes in the odor, consistency, color of the samples in addition to the presence of threads, clots or precipitates according to (20). Diagnosing of the clinical cases of mastitis was done according to the cardinal manifestations of udder inflammation, including heat, swelling, redness, pain, and edema in the mammary gland, in addition to the clinical manifestations associated with mastitis, including increase in heart rate/min, respiratory rate/min, rectal temperature and rumenal movements/5min. The number of infected animals with clinical mastitis in this study was 85 animals while the number of control group was 50 animals.

California mastitis test (CMT)

The samples of milk were mixed gently, then the CMT was conducted by adding equal quantities of CMT reagent and milk in a paddle. The reaction occurred after mixing the mixture in a gently circular motion of the paddle. Finally, the results were recorded according to (20).

Examination of the milk samples with Ultrasonic milk analyzer (Eko-milk Analyzer)

The milk samples were examined directly within two hours after collection by using Ultrasonic Milk Analyzer machine (Eon-Trading company/ Bulgaria). The machine estimated the EC of the milk samples, the percentage of total solids, lactose, protein, fat, pH as well as the density of the milk samples.

Somatic cell count

The milk samples were mixed thoroughly by vortexing and the cream was dispersed throughout the specimen. This was followed by the spreading of, 0.01 ml of milk over 1 sq.cm area on a slide (1*3 inch). The slides were dried over a flat surface without heating, then the slides were stained with Newman-Lampert stain to show the leukocytes. The microscopic examination was carried out carefully to estimate the number of somatic cells by multiplying the average of somatic cells in 30-50 fields by the microscopic

factor by 100. This method gave the SCC per milliliter of milk (20).

Bacterial culture and identification

The number of milk samples cultured was 304(85 samples from clinical cases and 219 samples from subclinical cases). Culturing of the milk samples was done on ordinary media, namely Blood agar, Nutrient agar, MacConkey agar (Himedia company, India) and incubated at 37°C for 24 hours. Following the development of bacterial colonies on these media, a slide was made from the colonies, then stained with Gram's stain. The bacterial colonies were subcultured on selective media such as Hoyles media for *Corynebacterium spp*, Edward's media for *Streptococcus spp*, Manitol salt agar and Staphylococcus medium No. 110 for *Staphylococcus spp* (Difco company, USA). Following that, the biochemical tests were carried out including Oxidase test, Methyl red test, Indole test, Citrate utilization test, Voges-proskauer test, Catalase test, Urate test, Nitrate reduction test, Sugar fermentation test and Coagulase test according to (21) to identify the different bacterial species. Fifty milk samples without bacterial isolates were considered as control.

Statistical analysis

Following the One-way analysis of variance (ANOVA) was the performance of the *post-hoc* test (Duncan) in the same program, which was utilized to compare the clinical cases, subclinical cases and control group for clinical signs, chemical analysis of milk samples and SCCs, while the differences in the prevalence of mastitis between several risk factors was appraised by utilizing two-sided Chi-square and Fischer's exact test in IBM-SPSS statistics version 19 program and the relative risk (RR) for the relationship among risk factors for mastitis by employing 2 x 2 tables in Epi-Info TM 7 software (version 7).

Results

According to the clinical signs it was found that 10.63% (85 out of 800) of the animals were infected with clinical mastitis, while 27.37% (219 out of 800) of them were infected with subclinical mastitis according to CMT (Table 1). The infected animals with the clinical cases of mastitis showed evidence of swelling of the infected quarters accompanied by pain, redness of the teat, and heat in addition to the presence of flakes, clots, purulent material or blood in the milk. All the clinical signs were present in one quarter. The results of CMT for the milk samples indicated that the degrees of reaction were (+) in 93 milk samples and (++) in 126 milk samples (Table 1). The results of statistical analysis of the clinical signs revealed a significant rise in the heart as well as respiratory rate/min and rectal temperature of the infected animals with clinical cases of mastitis in comparison with control, while there was a significant reduction in the rumenal movement/5 min of the clinical mastitis infected animals in comparison with control (Table 2). In the current study, there was a considerably increased SCC in the milk samples of infected animals with subclinical and clinical cases of the disease in comparison with the control group (Table 3).

The chemical composition of the milk samples of the infected animals with subclinical and clinical cases of mastitis was statistically analyzed and revealed the presence of a significant decrease in the amount of overall solids, lactose, protein, fat, and density of the milk samples of the infected animals with subclinical and clinical forms of mastitis in comparison with their levels in the control group, while there was a significant rise in the levels of electrical conductivity (EC) and pH of the milk samples of the infected animals with subclinical and clinical forms of mastitis compared to their levels in the control group (Table 4).

Table 1: Results of CMT for milk samples of buffaloes infected with subclinical cases of mastitis (n=800)

Type of infection	Reaction degree	No. of positive samples	Infection rate (%)
Subclinical infection	+	93	
Subclinical infection	++	126	27.37
Total		219	

Table 2: The clinical signs of the clinical, subclinical cases of mastitis and control group in buffaloes (mean±SE)

Clinical symptoms	Clinical mastitis 85 animals	Subclinical mastitis 219 animals	Control group 50 animals
Heart rate/min	96.4 ± 4.21 [a]	76.8 ± 6.24 [b]	73.8 ± 4.32 [b]
Respiratory rate/min	79.4 ± 5.37 [a]	35.4 ± 4.62 [b]	33.8 ± 3.53 [b]
Rumenal movements/5min	1.8 ± 0.7 [b]	3.2 ± 0.3 [a]	4.4 ± 0.2 [a]
Rectal temperature °C	41.1 ± 0.31 [a]	38.7 ± 0.24 [b]	38.2 ± 0.53 [b]

Mean values ± Standard error (S.E.) significantly different ($P<0.05$) between buffaloes, and those of healthy status are labeled with different superscript letters ([a],[b] or [c]).

Table 3: Number of somatic cells/ml of milk samples of buffaloes infected with clinical, subclinical cases of mastitis and control group (mean±SE)

Age	Milk samples of Clinical cases No. of Somatic cells/ml($x10^3$) (85 samples)	Milk samples of Subclinical cases No. of Somatic cells/ml ($x10^3$) (219 samples)	Milk samples of Control group No. of Somatic cells/ml ($x10^3$) (50 samples)
4-5 years	1284 ± 36.2 [a]	324 ± 10.4 [b]	74 ± 4.2 [c]
6-7 years	1458 ± 29.6 [a]	336 ± 12.8 [b]	79 ± 6.8 [c]
8-9 years	1648 ± 42.7 [a]	347 ± 17.3 [b]	86 ± 8.4 [c]
10-11 years	1795 ± 48.3 [a]	385 ± 16.8 [b]	92 ± 6.7 [c]

Mean values ± Standard error(S.E.) significantly different (P<0.05) between buffaloes, and those of healthy status are labeled with different superscript letters.

Table 4: Results of chemical analysis of milk samples of buffaloes infected with clinical, subclinical cases of mastitis and control group (Mean ± S.E)

Age (year)	Milk components	Milk samples of Clinical cases (85 samples)	Milk samples of Subclinical cases (219 samples)	Milk samples of Control group (50 samples)
4-5	Total solids %	13.14 ± 1.16 [b]	13.23 ± 1.21 [b]	16.45 ± 1.32 [a]
	Lactose %	3.47 ± 0.26 [b]	3.52 ± 0.41 [b]	4.71 ± 0.27 [a]
	Protein %	3.92 ± 0.32 [b]	3.88 ± 0.25 [b]	5.32 ± 0.22 [a]
	Fat %	5.74 ± 0.83 [b]	5.62 ± 0.95 [b]	7.88 ± 1.24 [a]
	pH	7.59 ± 0.32 [a]	7.64 ± 0.46 [a]	6.94 ± 0.30 [b]
	Density gm/ml	1.025 ± 0.006 [b]	1.022 ± 0.004 [b]	1.038 ± 0.002 [a]
	Electrical conductivity	8.26 ± 0.26 [a]	6.14 ± 0.014 [b]	3.12 ± 0.004 [c]
6-7	Total solids %	12.37 ± 1.54 [b]	12.74 ± 1.72 [b]	16.37 ± 1.64 [a]
	Lactose %	3.35±0.31 [b]	3.63 ± 0.2 [b]	4.64 ± 0.35 [a]
	Protein %	3.74 ± 0.32 [b]	3.64 ± 0.13 [b]	5. 29 ± 0.38 [a]
	Fat %	5.28 ± 0.94 [b]	5.47 ± 0.84 [b]	7.58 ± 1.31 [a]
	pH	7.64 ± 0.42 [a]	7.52 ± 0.36 [a]	6.88 ± 0.21 [b]
	Density gm/ml	1.029 ± 0.003 [b]	1.027 ± 0.002 [b]	1.036 ± 0.003 [a]
	Electrical conductivity	8.36 ± 0.031 [a]	6.26 ± 0.022 [b]	3.8 ± 0.005 [c]
8-9	Total solids %	12.24 ± 1.24 [b]	12.53 ± 1.66 [b]	16.62 ± 1.43 [a]
	Lactose %	3.52 ± 0.18 [b]	3.64 ± 0.32 [b]	4.58 ± 0.27 [a]
	Protein %	3.58 ± 0.21 [b]	3.66 ± 0.14 [b]	4.75 ± 0.26 [a]
	Fat %	5.11 ± 0.25 [b]	5.23 ± 0.18 [b]	6.68 ± 0.32 [a]
	pH	7.64 ± 0.17 [a]	7.52 ± 0.31 [a]	6.82 ± 0.11 [b]
	Density gm/ml	1.026 ± 0.004 [b]	1.027 ± 0.002 [b]	1.035 ± 0.002 [a]
	Electrical conductivity	8.16 ± 0.31 [a]	6.26 ± 0.018 [b]	4.42 ± 0.007 [c]
10-11	Total solids %	12.91 ± 1.36 [b]	13.20 ± 1.42 [b]	16.42 ± 1.37 [a]
	Lactose %	3.44 ± 0.21 [b]	3.56 ± 0.16 [b]	4.48 ± 0.41 [a]
	Protein %	3.71 ± 0.18 [b]	3.86 ± 0.22 [b]	4.66 ± 0.34 [a]
	Fat %	5.76 ± 0.42 [b]	5.78 ± 0.32 [b]	6.59 ± 0.42 [a]
	pH	7.56 ± 0.22 [a]	7.38 ± 0.16 [a]	6.78 ± 0.14 [b]
	Density gm/ml	1.022 ± 0.006 [b]	1.025 ± 0.004 [b]	1.033 ± 0.003 [a]
	Electrical conductivity	8.21 ± 0.21 [a]	6.16 ± 0.018 [b]	5.42 ± 0.006 [c]

Mean values ± Standard error (S.E.) significantly different (P<0.05) between buffaloes, and those of healthy status are labeled with different superscript letters.

Based on the clinical signs and CMT for the milk samples of the buffaloes, the occurrence of mastitis was significantly higher among 10 to 11-year-old buffaloes at 62.60% relative risk(RR): 6.796 times, Confidence Interval (CI): 4.088-11.300] than eight to nine-year-old, six to seven-year-old and four to five-year-old buffaloes, which were 41.22%, 24.13% and 9.21% respectively (Table 5).The prevalence of mastitis was considerably elevated

among the third trimester of lactation period 54.93% (RR: 4.154 times, CI: 2.964- 5.822) compared to the second and first trimesters of lactation period, which were 40.17% and 13.22% respectively (Table 5). The occurrence of mastitis was considerably elevated among outdoor feeding animals 49.31% (RR: 2.028 times, CI:1.652- 2.490) compared to the indoor feeding animals, which were 24.30% (Table 6). The prevalence of mastitis was considerably higher among large herd sizes (> 30 buffaloes/herd) 44.84% (RR: 2.273, CI: 1.713-3.015) compared to small herd sizes (<10 buffaloes/herd), which were 19.72%. The occurrence of mastitis was considerably elevated among the soil-floored bedding buffaloes 61.72% (RR: 3.372 times, CI: 2.524-4.505) compared to the cement and sand-floored bedding buffaloes which were 25.00%, 18.30% respectively (Table 6). The occurrence of mastitis was considerably elevated in the northern, southern parts of Mosul city, 66.97%, 48.57% respectively (RR: 5.357, 3.885 times respectively, CI: 3.253-8.822 and 2.334-6.466 respectively) than the central, eastern and western parts of Mosul city, which were 17.64%, 14.51% and 12.5% respectively (Table 7). The prevalence of the disease was substantially elevated in Spring and Winter seasons, 66.24%, 40.75% respectively (RR: 6.219, 3.826 times respectively, CI: 3.981-9.715 and 2.401-6.098 respectively) than Autumn and Summer seasons, which were 23.49%,10.65% respectively (Table 8).

The numbers, types and percentages of the bacterial isolates from clinical cases of mastitis were: *Staphylococcus aureus,* 22 isolates (25.88%), *Streptococcus agalactiae,* 16 isolates (18.82%), *Streptococcus pyogenes,* 14 isolates (16.47%), *Corynebacterium bovis,* 12 isolates (14.11%), *Escherichia coli,* 9 isolates (10.58%), *Pseudomonus aeruginosa,* 6 isolates (7.05%) and *Pasteurella multocida,* 6 isolates (7.05%) (Table 9). The numbers, types and percentages of the bacterial isolates from subclinical cases of mastitis were: *Staphylococcus chromogens,* 32 isolates (14.61%), *Staphylococcus xylosus,* 28 isolates (12.78%), *Streptococcus agalactiae,* 26 isolates (11.87%), *Streptococcus dysagalactiae,* 25 isolates (11.41%), *Streptococcus uberis,* 22 isolates (10.04%), *Proteus vulgaris,* 22 isolates (10.04%), *Klebsiella pneumonae,* 21 isolates (9.58%), *Escherichia coli,* 18 isolates (8.21%), *Corynebacterium bovis* 16 isolates (7.30%) and *Passteurella multocida,* 9 isolates (4.10%) (Table 10).

Table 5: Risk of animal factors associated with occurrence of mastitis based on California mastitis test (CMT)

Factors	Number	California Mastitis test			
		No. of positive (%)	Relative Risk (RR)	95% Confidence Interval (CI)	P-value
Age					
4-5 years	152	14 (9.21) [a]	1		
6-7 years	174	42 (24.13) [b]	2.620	1.490-4.608	0.0003
8-9 years	228	94 (41.22) [c]	4.476	2.654-7.548	0.0001
10-11 years	246	154 (62.60) [d]	6.796	4.088-11.300	0.0001
Lactation period					
First trimester	242	32 (13.22) [a]	1		
Second trimester	234	94 (40.17) [b]	3.037	2.122-4.348	0.0002
Third trimester	324	178 (54.93) [c]	4.154	2.964-5.822	0.0001

Values significantly different (P<0.05) between animal factors are labelled with different superscript letters.

Table 6: Risk of the management factors associated with occurrence of mastitis based on California mastitis test (CMT)

Factors	number	California Mastitis test			
		No. of positive (%)	Relative Risk	95% Confidence Interval	P- value
Husbandry					
Indoor feeding	362	88 (24.30) [a]	1		
Outdoor feeding	438	216 (49.31) [b]	2.028	1.652-2.490	0.0001
Herd size					
<10 Buffalos	218	43 (19.72) [a]	1		
>30 Buffaloes	582	261 (44.84) [b]	2.273	1.713-3.015	0.0001
Type of floor bedding					
Sand floored	224	41 (18.30) [a]	1		
Cement floored	252	63 (25.00) [b]	1.365	0.962-1.937	0.077
Soil floored	324	200 (61.72) [c]	3.372	2.524-4.505	0.0001

Values significantly different (P<0.05) between management factors are labelled with different superscript letters.

Table 7: Risk of the regional factors associated with occurrence of mastitis based on California mastitis test (CMT)

Factors	No. of buffaloes	California Mastitis test			
		No. of positive (%)	Relative Risk	95% Confidence Interval	P- value
Western	112	14 (12.5) [a]	1		
Eastern	124	18 (14.51) [a]	1.161	0.606-2.224	0.651
Central	136	24 (17.64) [b]	1.411	0.767-2.597	0.042
Southern	210	102 (48.57) [c]	3.885	2.334-6.466	0.0001
Northern	218	146 (66.97) [d]	5.357	3.253-8.822	0.0001

Values significantly different (P<0.05) between regional factors are labelled with different superscript letters ([a, b, c or d]).

Table 8: Risk of the seasonal factors associated with occurrence of mastitis based on California mastitis test (CMT)

Factors	No. of buffaloes	California Mastitis test			
		No. of positive (%)	Relative Risk	95% Confidence Interval	P- value
Summer	169	18 (10.65) [a]	1		
Autumn	183	43 (23.49) [b]	2.206	1.326-3.670	0.001
Winter	211	86 (40.75) [c]	3.826	2.401-6.098	0.0001
Spring	237	157 (66.24) [d]	6.219	3.981-9.715	0.0001

Values significantly different (P<0.05) between seasonal factors are labelled with different superscript letters ([a, b, c or d]).

Table 9: Types and frequencies of different bacterial species isolated from clinical Cases of mastitis in buffaloes

Type of animals	Bacterial species	4-5 years	6-7 years	8-9 years	10-11 years	No. of isolates	Isolation Percentage (%)
Buffaloes	*Staphylococcus aureus*	2	4	7	9	22	25.88
	Streptococcus agalactiae	1	2	6	7	16	18.82
	Streptococcus pyogenes	2	2	4	6	14	16.47
	Corynebacterium bovis	1	2	4	5	12	14.11
	Escherichia coli	1	1	2	5	9	10.58
	Pseudomonus aeruginosa	-	1	2	3	6	7.05
	Pasteurella multocida	1	1	2	2	6	7.05
	Total	8	13	27	37	85	100.00

Table 10: Types and frequencies of different bacterial species isolated from subclinical cases of Mastitis in buffaloes

Type of animals	Bacterial species	4-5 years	6-7 years	8-9 years	10-11 years	No. of isolates	Isolation Percentage (%)
Buffaloes	*Staphylococcus chromogens*	1	3	10	18	32	14.61
	Staphylococcus xylosus	1	3	10	14	28	12.78
	Streptococcus agalactiae	1	4	7	14	26	11.87
	Streptococcus dysagalactiae	1	4	8	12	25	11.41
	Streptococcus uberis	-	3	6	13	22	10.04
	Proteus vulgaris	-	2	6	14	22	10.04
	Klebsiella pneumonae	-	2	7	12	21	9.58
	Escherichia coli	1	3	6	8	18	8.21
	Corynebacterium bovis	1	3	4	8	16	7.30
	Pasteurella multocida	-	2	3	4	9	4.10
	Total	6	29	67	117	219	100.00

Discussion

In this paper, the total prevalence of the clinical mastitis in buffaloes was 10.62% based on clinical signs, while the total occurrence of SCM was 27.37% based on CMT. These outcomes were lower compared with previous outcomes reported by (22) who stated that the occurrences of the clinical and subclinical cases of mastitis in buffaloes in

Missan City/Iraq were 25.17% and 31.94% respectively, according to the clinical signs, bacterial culture and identification techniques. The prevalence of SCM in this study was higher compared to the outcomes reported by (23) who reported the occurrences of SCM in buffaloes were 7.05% based on CMT.

The statistical analysis of the clinical signs revealed that the infected buffaloes with clinical cases of mastitis had significantly higher heart and respiratory rate/min, rectal temperature, but they had significantly lower values of rumenal movements/5min compared to control and associated with cardinal signs of inflammation such as swelling, heat, redness, discomfort, and edema in the infected quarters with abnormally discolored milk and existence of threads, flakes, and clots in the mastitic milk and this result is compatible with (1,6,13).

A considerable increase was noted in the SCC in the clinical and subclinical instances of the disease in the current study in comparison with the control animals. In the SCM cases the SCC was higher than 200.000 somatic cells/ml of milk, which suggested infection with SCM according to (16-18) who reported that healthy buffalo milk had < 100.000 somatic cells/ml of milk and the buffalo milk having > 200.000 somatic cells/ml were considered subclinically infected, while in the clinical cases of mastitis the SCC was higher, ranging between 1.284.000 ± 36.2 to 1.795.000 ± 48.3 and this result suggested the clinical infection of mastitis according to (23).

The SCC of milk is the true index of intramammary infections. The somatic cells in the milk are of two types, including the blood leukocytes and sloughed epithelial cells of the mammary gland tissues. The epithelial cells are found normally in milk due to the natural breakdown and repair function in the mammary gland tissues, while the leukocytes influx from the blood to the milk, which are drawn by chemical releases from damaged tissues in the mammary gland. The majority of the somatic cells are leukocytes, including neutrophils, lymphocytes and macrophages. The epithelial cells range from 0 - 7 % of the SCC but the main elevation in SCC occurs because of the flow of neutrophils into the milk (24).

The SCC rises along with the seriousness of the mammary tissue inflammation (6). The SCC is an indicator of the udder inflammation which is used for monitoring udder health and milk quality at quarter, cow, herd and general animal population levels. In the current study the SCC in the milk of old age buffaloes was higher than the SCC of the milk of young age buffaloes and this result suggested the higher occurrence of mastitis in old age buffaloes compared to young age buffaloes due to the higher susceptibility of the old age buffaloes to teat abrasions compared to the young age buffaloes (7).

In the current study there is a substantial decline in lactose levels in the milk samples of the clinical and subclinical forms of mastitis compared to the milk samples of the control group and this result agrees with the that of (24), who reported a considerable decline in the lactose levels in the clinical and subclinical cases of mastitis, which may be attributed to the reduction in the synthetic activities of the mammary gland's epithelial cells.

In the current study, there is a notable decline in the protein content in the milk samples of the clinical and subclinical cases of mastitis in comparison with the milk samples of the control group and this result is compatible with the findings of (25), who revealed a substantial reduction in the protein contents of the mastitic milk, possibly because of the breakdown of milk protein in cases of clinical and SCM, The milk from clinical or subclinical infected animals was found to have an elevated proteolytic enzyme activity (plasmin), and this enzyme caused substantial damage to the milk casein in the mammary gland before the milk was collected from the animal.

This study noted a considerable reduction in the levels of fat in the milk samples of the clinical and subclinical cases of mastitis in comparison with the milk samples of the control, which agrees with the reports of (25) who found significant reduction in the levels of fat in the milk samples of infected animals and this may be attributed to weakened synthesis and the secretion function of the mammary gland's epithelial cells. Furthermore, it may be attributed to the breakdown of fat due to the effects of lipase enzyme, which attacks the triglycerides in the milk and releases free fatty acids that may produce a rancid off-flavor from the mastitic milk (23).

This current study also witnessed a noticeable rise in the pH values in the milk samples of the clinical and subclinical forms of mastitis in comparison with the milk samples of the control. This result is in agreement with the results reported by (24), that the pH values increase significantly in mastitic milk samples in comparison with normal milk samples, probably because of the increased permeability of the udder tissue to blood components leading to their higher values in the milk, such as increased influxes of bicarbonate ions from the blood stream into the milk. The movement of alkaline salts from the blood to the milk makes the milk more alkaline, showing pH values over 7 and this result is compatible with (26). Besides, (27) mentioned that the elevated SCC in the mastitic milk led to elevation in the pH values of the milk, which may be due to increase in alkaline salt production in the udder of the infected animals with mastitis.

The current study witnessed a considerable increase in the EC of the milk samples of clinically and subclinically-infected animals in comparison with the control animals. This result was concurring with the findings of (28) who reported that the range of the EC of healthy milk was 3.0-5.48 (melli Seimens) mS/cm and the range of EC of the subclinical mastitis (SCM) was 5.50-6.50 mS/cm, while the range of EC of the clinical mastitis was 6.5-8.5 mS/cm. Furthermore, (24) revealed considerable increases in the EC

of milk samples of mastitis-infected animals. The rise in the EC may be attributed to the increased levels of Na^+ and Cl^- ions and reductions in the concentrations of lactose and K^+ions. There are two factors that cause these alterations in the ionic levels in mastitic milk namely the damage of the active ion pumping system, and increased permeability of the blood capillaries. These changes in the levels of Na^+, Cl^- and K^+ ions in the milk during mastitis lead to higher EC of the milk. The EC of the milk has a positive correlation with the SCC, so the detection of mastitis can be confirmed by the measurement of the EC of the milk.

Moreover, considerably higher occurrence of mastitis was noted in this study among old age buffaloes of 10-11 years and 8-9 years in comparison with medium age buffaloes of 6-7 years, and young age buffaloes of 4-5 years, and this result is compatible with (7,24) who states that the maximum occurrence of mastitis occurs in buffaloes aged 11 years and older 72.72%, while the lowest occurrence of mastitis in buffaloes occurs in buffaloes aged 5-6 years 3.46 - 4.25%.

The existence of mastitis was found in this study to be significantly elevated in the third trimester of lactation period, and this result mirrors that of (24), who reported increased occurrence of mastitis in the third trimester compared to the first and second trimesters of lactation period, and this may be attributed to decreased host immune defense capabilities during the third trimester of lactation period in buffaloes, in addition to more susceptibilities of the udder in the third trimester of lactation to environmental and contagious pathogens. Furthermore, (29) reported the maximum occurrence 71.43% of SCM at the third trimester of lactation period in comparison with mid and first trimesters.

The prevalence of mastitis in the current study in outdoor feeding animals was higher compared to the indoor feeding buffaloes, and this may be attributed to higher susceptibility of the outdoor feeding animals to abrasions and injuries of the teats and the udder tissues during grazing compared to the indoor feeding animals and this result is compatible with (27). Besides, the susceptibility of the outdoor feeding animals to the environmental and contagious pathogens is higher than the indoor - feeding animals.

In this present study, the occurrence of mastitis was more predominant in the large size herds (> 30 buffaloes) than the small size herds (< 10 buffaloes), and this may be attributed to the overcrowding of the animals in the herd and increased transmission of contagious pathogens from the infected animals to the healthy animals which reflects incorrect farm management, general discomfort and mammary stress leading to the production of poor milk quality (6).

The occurrence of mastitis in the present work was determined to be considerably higher in animals reared in soil-floored farms, whereas lower occurrence was reported in buffaloes reared in cement and sand floored farms, and this result is compatible with (2) who reported highest occurrence of mastitis in animals reared on soil-floored farms, and lower occurrence in animals reared on cement and sand floored farms with 29% and 27% respectively, and this may be attributed to the fact that the soil floored farms provide favorable conditions for bacterial growth more than the cement and sand floored farms. This is because in the soil floored farms, the teats of the animals are exposed to the pathogenic bacteria, leading to increased probabilities of mastitis in buffaloes.

The occurrence of mastitis in the present research was determined to be higher in the northern and southern parts of the Mosul city/Iraq, than the central, eastern and western parts of the city, and this may be attributed to the intensively managed buffaloes and high stocking density, in addition to dirty bedding, poor ventilation, and infected utensils in the northern and southern parts of the city, and this result is compatible with (29) who also reported highest occurrence of mastitis in high intensively managed animals.

The occurrence of mastitis in this present study was considerable higher in Spring and Winter seasons than in Autumn and Summer seasons, and this result agrees with (30,31) who revealed highest prevalence of mastitis during Spring and Winter seasons, which may be due to high rainfall and high humidity in Spring and Winter seasons in Mosul city/Iraq, during which the chances of contamination of the teats and udders with mud and infected manures are higher than during other seasons and provides a favorable conditions for substantial bacterial growth.

This present study found that the highest occurrence of bacterial infections in the clinical cases of mastitis was *Staphylococcus aureus* followed by *Streptococcus agalactiae* and *Streptococcus pyogenes*. The current study also found that the predominant bacteria in clinical cases of mastitis were *Staphylococcus aureus* and this result is compatible with (32,33) who noted that the *Staphylococcus aureus* were the predominant bacteria 38%, 54.55% respectively. Besides, (34) reported higher occurrence of *Staphylococcus aureus* in clinical cases of mastitis, which was 48.57%, while (35,36) reported that the *coagulase negative Staphylococci* (CNS) were the most predominant bacterial species in clinical cases of mastitis in buffaloes.

In the case of the current study, the highest occurrence of bacterial species in the subclinical cases of mastitis was *Staphylococcus chromogens* 32 (14.61%), followed by *Staphylococcus xylosus* 28 (12.78%), *Streptococcus agalactiae* 26 (11.87%) and *Streptococcus dysagalactiae* 25 (11.41%). In the subclinical (SC) cases of mastitis, the predominant bacteria were *Staphylococcus chromogenes* 32 (14.61%) and this finding was compatible with (37) who mentioned that the commonest isolates from the milk of buffaloes were *Staphylococcus chromogenes*, *Staphylococcus hyicus* and *Streptococcus uberis*, while (2) reported that the prevalence of *Streptococcus agalactiae*

and *Streptococcus dysagalactiae* from subclinical cases of mastitis in buffaloes were 3.12%, and 0.62% respectively. Furthermore, (36) mentioned that the occurrence of *Streptococcus agalactiae* was 4.4%, *Streptococcus dysagalactiae*, 5.6%, while (37) mentioned that the prevalence of *Streptococcus uberis* was 4.28%, and *Streptococcus dysagalactiae* was 11.42% in the SC cases of mastitis.

Conclusion

Mastitis leads to a significant reduction in the levels of total solids, lactose, protein, fat, density, and a significant rise in the levels of pH values and electrical conductivity of the milk samples of clinical and subclinical cases of mastitis. The occurrence of SCM was higher compared to the clinical cases in buffaloes, and the occurrence of mastitis in old age buffaloes was higher than the young age buffaloes.

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Conflict of interest

The authors declare no conflicts of interest.

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التهاب الضرع السريري وتحت السريري في الجاموس في منطقة الموصل، العراق

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الخلاصة

تستهدف هذه الدراسة التحري عن نسبة حدوث التهاب الضرع السريري وتحت السريري في الجاموس، ولمعرفة التغيرات في مكونات الحليب، وللتحري عن عدد من عوامل الخطورة الوبائية لحدوث حالات التهاب الضرع وللكشف عن الأنواع الجرثومية المصاحبة لحدوث المرض. تم في هذه الدراسة جمع ٨٠٠ عينة حليب جاموس عشوائيا من حيوانات بأعمار مختلفة في مدينة الموصل / العراق وتم فحصها باستخدام اختبار كاليفورنيا وجهاز تحليل الحليب الصوتي. واعتمادا على الأعراض السريرية واختبار كاليفورنيا أظهرت النتائج بأن نسبة انتشار حالات التهاب الضرع السريري وتحت السريري كانت ١٠,٦٢% و ٢٧,٣٧% على التوالي. وظهرت زيادة كبيرة في أعداد الخلايا الجسمية / مل من الحليب في كلا حالتي التهاب الضرع السريري وتحت السريري. وشملت عوامل الخطورة الثلث الثالث من فترة الحلب والأعمار الكبيرة ١٠-١١ سنة والحيوانات ذات التغذية الخارجية والحقول التي تحوي > ٣٠ جاموسة /حقل والأجزاء الشمالية والجنوبية من مدينة الموصل ومواسم الربيع والشتاء. وفي الحالات السريرية لالتهاب الضرع كانت نسبة الإصابة بالمكورات العنقودية الذهبية ٢٥,٨٨% والمكورات السبحية الحليبية ١٨,٨٢% والمكورات السبحية القيحية ١٦,٤٧% وجراثيم الوتديات البقرية ١٤,١١% وجراثيم الاشريشيا القولونية ١٠,٥٨% وجراثيم الزوائف الهوائية ٧,٠٥% وجراثيم الباستوريلا ملتوسيدا ٧,٠٥%. أما في الحالات تحت السريرية لالتهاب الضرع كانت نسبة الإصابة بالمكورات العنقودية الصبغية ١٤,٦١% والمكورات العنقودية الزايلوسية ١٢,٧٨% والمكورات السبحية الحليبية ١١,٨٧% والمكورات السبحية غير الحليبية ١١,٤١% والمكورات السبحية الاوبرسية ١٠,٠٤% وجراثيم بروتيس فولكارس ١٠,٠٤% وجراثيم الكليبسيلا الرئوية ٩,٥٨% وجراثيم الاشريشيا القولونية ٨,٢١% وجراثيم الوتديات البقرية ٧,٣٠% وجراثيم الباستوريلا ملتوسيدا ٤,١٠%. أستنتج من هذه الدراسة بأن حالات التهاب الضرع تؤدي الى انخفاض معنوي في مستويات المواد الصلبة الكلية واللاكتوز والبروتين والدهن وكثافة الحليب وارتفاع معنوي في مستوى الأس الهيدروجيني ودرجة التوصيل الكهربائي لعينات الحليب المصابة بالتهاب الضرع السريري وتحت السريري.