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RESEARCH ARTICLE

PREVALENCE OF GRAM POSITIVE BACTERIA IN BUFFALO MASTITIS IN IRAQ

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Abstract

The present study was designed to determine the buffalo and quarter-wise prevalence of mastitis with their bacterial etiological agents, we collected 332 milk samples from 83 lactating buffaloes for this purposes, the animals examined clinically as well as by CMT, bacteriological examination and VITEK identification technique, overall prevalence of mastitis in buffaloes was 44(53.01%) but in quarters test 93(28.01%). Clinical mastitis prevalence in buffaloes was 10(12%) and subclinical 34(41%) , while in quarters clinical infection was 14(4.2%) and subclinical infection was 79(23.8%), also higher prevalence recorded in hindquarters compared to those of forequarters in both clinical mastitis (RA 1(7.1%) , LA(0.0%) , RP 7(50%) , LP 6(42.9%)) and subclinical mastitis (RA 10(12.7%) , LA 8(10.1%) , RP 32(40.5%) , LP 29(36.7%)). Bacterial isolation reveled in clinical quarters S.sciuri 2(14.3%), S.agalactiae 3(21.4%) , S.dysagalactiae 1(7.1%) , S. uberis 4(28.6%) , S. canis 3(21.4%) , S. equi 1(7.1%) , L.garvaiae 1(7.1%), but gram negative bacteria included : Enterobacteriacae spp. 0(0.0%) and Other gram negative 2(14.3%). In subclinical quarters the following organisms: 5(6.3%) S. aureus 1(1.3%) S.epidermidis 5(6.3%) S. sciuri 4(5.1%) S. lentus 14(17.7%) S. chromogens 3(3.8%) S. haemolyticus and 4(5.1%) S. xylosus, S. agalactiae 6(7.6%), S. uberis 16(20.3%), S. thoraltensis 3(3.8%), S. equi 1(1.3%), E. faecium 2(2.5%), E. gallinarum 3(3.8%), E. casseliflavus 4(5.1%), L. garvaiae and K. rosea 1(1.3%) for each and A. otitis 2(2.5%) while Enterobacteriacae spp. 13(16.5%) and other gram negative bacteria 1(1.30%). Among Staphylococcus spp. the most predominant bacteria was S. chromogens in subclinical infection and S. sciuri was the only one isolated in clinical infection. But among Streptococcus spp. in both clinical and subclinical infection S. uberis represent the most common isolated bacteria followed by S. agalactiae. From Gram negative bacteria, Enterobacteriacae spp. was the most predominant in subclinical infection.

INTRODUCTION

Population Growth rate depend on a large demand and heavy reliance on milk and milk products in both wealthy countries and poorest communities in the world **(Ahmed et al. 2002; Beghin, 2006; Fazaa, 2007).** In fact, Milk plays important role in human life, primarily as a source of perfect food and has always occupied a significant position in the feeding style **(Ryser, 1998).**

Nevertheless, in our country buffaloes suffered from neglecting for many years and affected by various factors that lead to severe decline in population and production **(Alsaedy, 2007)**. Many diseases present among Iraqi buffaloes and mastitis considered as the most important one from the economic point of view **(Abdul Razak, 1982).** while every country even the developed ones suffer a huge financial losses due to this disease , which is considered as a global problem because it adversely affects animal health, and cause a great deterioration in milk quality **(Singh and Bansal, 2004 ; Sharma et al., 2007 ; Sharif et al., 2009)**. Besides, its eradication was very difficult as a result of unavailability of preventive vaccination. Consequently, the buffalo become unproductive and once a quarter of the udder is lost this will lead to animal culling from the herd **(Abdul Razak, 1982).**

For all these reasons, dairy industry is facing a great set back **(Shahid et al., 2011).** According to public health and food safety experts each year millions of illnesses throughout the world can be traced to food borne pathogens (**Oliver et al., 2005).**Raised from consuming milk from affected animal that harbor organisms potentially pathogenic for humans (zoonosis) **(Muhammad et al., 1995; Sharif and Ahmad, 2007).** So by keeping these view in mind we planning to study buffalo bacterial mastitis prevalence in Baghdad city/ Abu-Ghraib, particularly that considered as a health problem for consumers.

MATERILS AND METHODES:-

- **Animals and milk samples collection**

The present study was conducted on (332) milk samples collected from (83) buffaloes in White gold village - Abu Gharib - Baghdad city during the period from April to September, 2013. The lactating buffaloes included in this study examined clinically to determine the type of mastitis and to detect the abnormal signs particularly on mammary gland. aseptically 10 -20 mL of milk were collected from quarters in a sterile screw capped vials labeled as Right anterior (RA), Right posterior (RP), Left anterior (LA) and Left posterior (LP), transferred in an insulated container with ice packs to the laboratory for applying California mastitis test (CMT) and bacterial culturing depending on **Coles (1986), Watts (1990)** and **Radostits et al. (2000)** instructions.

- **California mastitis test (CMT)**

By mixing equal volume of milk samples with CMT reagent (Immucell/ USA) in receptacles of a white plastic paddle **,** with gentle circular motion for about (10) seconds, the results were scored as (-ve , +1, +2, and +3) depending on **Schalm et al. (1971)**

- **Bacteriological culturing**

Inoculated 0.5 ml of each milk samples into 5 ml brain heart infusion broth tubes incubated at 37 C° for 24-48 h. **(Bibek et al. 2012)** then streaked a loopful from each tube onto 5% sheep blood agar and incubated at 37 C° examined for bacterial growth after 24- 48 h. **(Quinn et al. 2004).** The presence of 6 or more bacterial colonies of the same type on the medium we considerd them to be significant and the samples were recorded as positive **(Quinn et al. 2004; Rahim and Jalal, 2010).** Purified on blood agar media then subcultured on selective media included Edward's media, mannitol salt agar and MacConkey agar, then primary and specific biochemical tests were done according to **Quinn et al. (2004)**

- **Bacterial identification by using VITEK technique**

In addition to conventional bacteriological and biochemical procedure we used a commercial identification kit of VITEK 2 system for Gram positive ID (BioMerieux/France)**.** After we determined the gram reaction of each isolates, we cultured them on TSAB by following the cultural requirement information in order to prepare the organisms suspension according to gram positive VITEK 2 system card then we placed the suspension tubes with the GP cards in VITEK2 compact cassette to start the filling process. Reading bar code, Sealing, Loading then Removing process. We displayed the results of isolates identification at the second day.

- **Statistical analysis**

Data statistical calculation and findings results were analyzed by the following tests: Binomial- test based on target of testing value 0.50 ,One-Sample Kolmogorov-Smirnov Test procedure compared the observed cumulative distribution function , Z - test based on difference between two proportions, Multiple Z - test based on difference among several proportions

RESULTS AND DISCUSSION:-

Mastitis is one of the most significant health problems of dairy herds because it leads to physical, chemical and bacteriological changes in the milk of dairy animals resulting inferior quality and quantity of produced milk **(Sharma et al. 2007a)**. Its prevalence in buffaloes in many developing countries did not well determined, leading to little information provided about this important disease (**Vajdi Hokmabad et al. 2011)**.

Table (1): Prevalence of mastitis in iraqi buffaloes

Comparison Significant (*)	No of Buffaloes examined	No of affected Buffaloes	%	No of quarters examined	No of affected Quarters	%
	83	44	53.01	332	93	28.01
C.S (*) P-value.	P=0.661 (NS)			P=0.000 (HS)		

(*) HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05
Binomial- test based on target of testing value 0.50

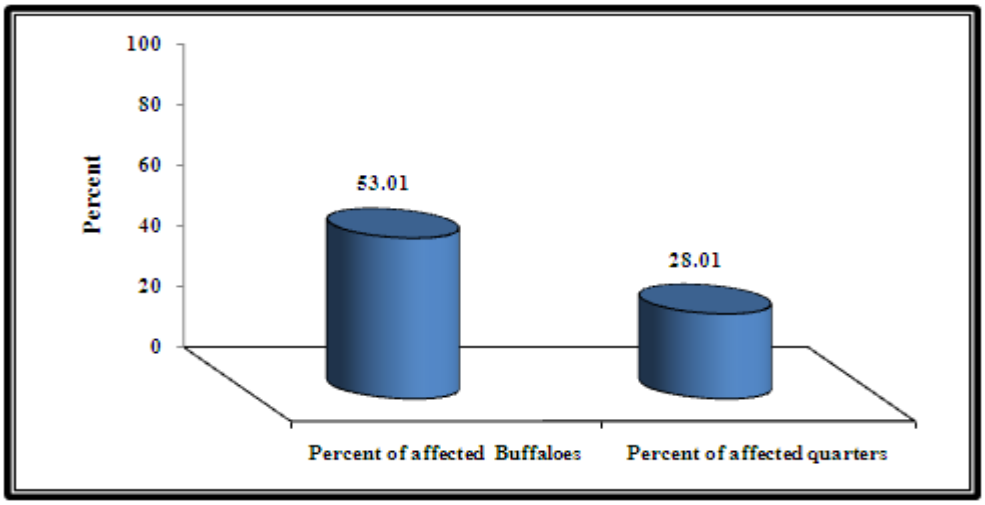


Figure (1): Percents prevalence of mastitis in iraqi buffaloes according to studied sample

In the present study, the total number of affected buffaloes with mastitis were 44(53.01%) out of 83 examined animals in White Gold Village / Abu Gharib while 93(28.01%) quarters were affected out of 332 total examined quarters, a highly significant difference in quarter wise infection but in animals wise was not significant **(Table & Fig. 1)** we recovered a higher prevalence compared with **Mustafa et al. (2011)** and **Vajdi Hokmabad et al. (2011**). This may attributed to the tighter teat sphincter of buffaloes **Mustafa et al. (2011)**. While our result indicated too wide of mastitis prevalence were accounted which may belong to bad management practice especially the overcrowding of reared animals in farmers houses in White Gold Village in addition to disappear of any kind of hygienic practices that should be followed in milking process which help in preventing the transmission of infection among the buffaloes then reducing the rate of infection in the herd these all due to low educational level of people there and absence of veterinary cares.

Table (2): Incidence of clinical and sub-clinical mastitis according to buffalo wise and quarter wise

Comparison Significant (*)	No of Buffaloes examined	No of Buffaloes with Clinical mastitis	%	No of Buffaloes with Subclinical Mastitis	%	No of quarter examined	No of quarter with Clinical mastitis	%	No of quarter with Subclinical Mastitis	%
	83	10	12	34	41	332	14	4.2	79	23.8
C.S (*) P-value.	P=0.000 (HS)			P=0.124 NS		P=0.000 HS			P=0.000 HS	

(*) HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05
Binomial- test based on target of testing value 0.50

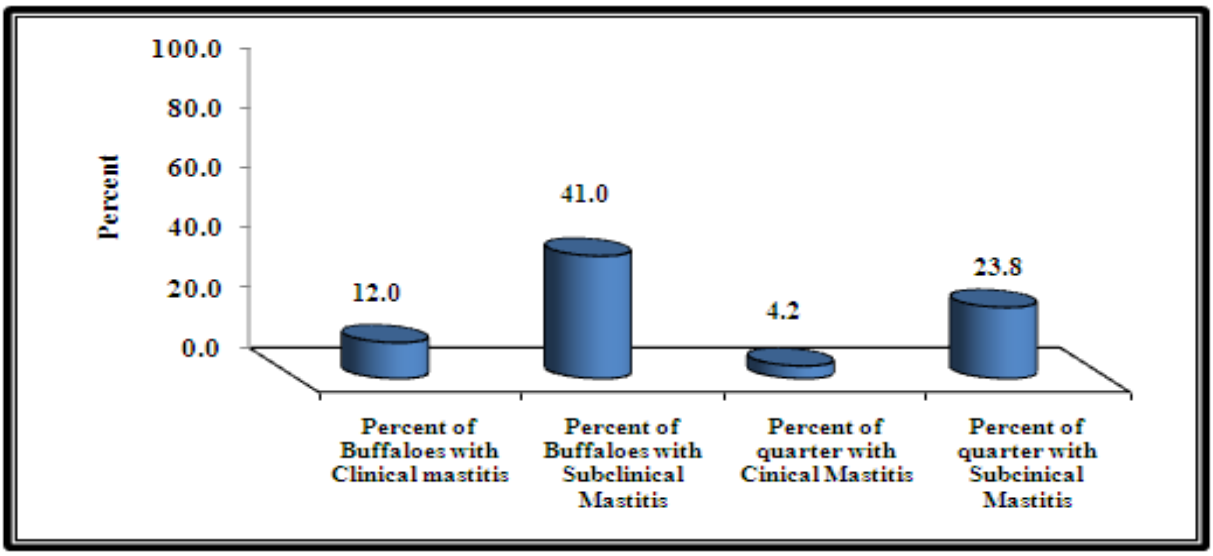


Figure (2): Percents incidence of clinical and sub-clinical mastitis according to buffalo wise and quarter

The results showed in **Table & Fig. (2)** Revealed that subclinical mastitis was higher than clinical mastitis according to animal wise (41%) , (12%) respectively and according to quarter wise infection was (23.8%) , (4.2%) respectively. We agreed with **Khan and Muhammad (2005)** results of high subclinical prevalence than clinical (27%), (4%) according to quarter wise. but we disagreed with **Bachaya et al. (2005)** and **Mustafa et al. (2011)** results , when they found higher rates of both clinical and subclinical mastitis compared with our results, in another hand **, Chavoshi and Husaini (2012)** and **Srinivasan et al. (2013)** found a lower rate of subclinical infection comparing to ours (9.5%) subclinical quarters and (26.20%) subclinical buffaloes respectively .

the high rate of subclinical more than clinical form of mastitis in buffaloes may returnd to easily diagnosed clinical infection based on apparent signs and symptoms with palpation of udder **(Sharif et al. 2009 a)** which encourage the animal's owner to seek for immediate treatment in order to prevent production loss. while subclinical infected animals remain showed no obvious symptoms and secreted apparently normal milk for long time during which pathogenic organisms transmitted in the herd, so subclinical mastitis stay always more dangerous than any other epidemiological form of this disease **(Bakken and Gudding , 1982)** and the disagreement with the other researchers may returned to differences in study areas and different animals rearing conditions in different country and traditions.

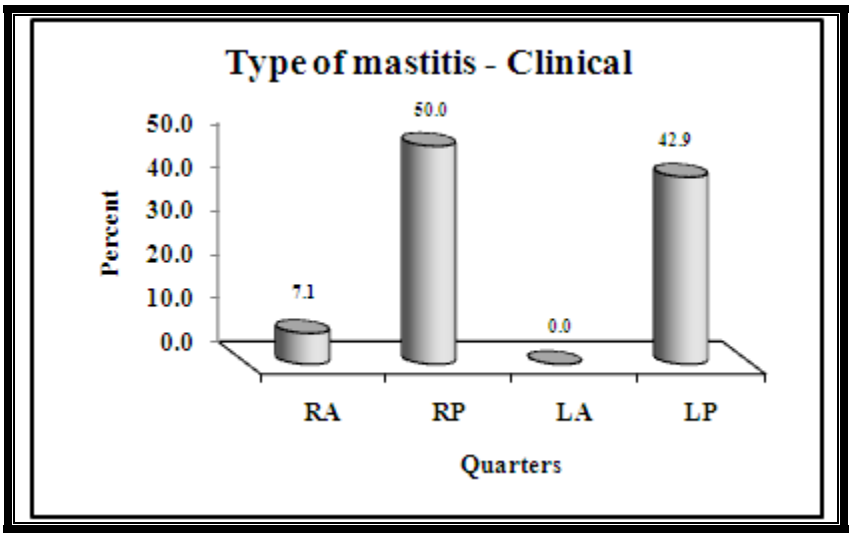
Table (3) Frequency and percents of clinical and subclinical mastitis according to quarter position

Comparison Significant (*)	Total no. of examined quarters	Type of mastitis	Total no. of affected quarters	Involvement of the quarters							
				RA		RP		LA		LP	
				No	%	No	%	No	%	No	%
	332	Clinical	14	1	7.1	7	50	0	0	6	42.9
		Subclinical	79	10	12.7	32	40.5	8	10.1	29	36.7
C.S (*) P-value.	Z= 7.268 P<0.01 (HS)			MZ $_{Clinical}$ = 10.915 P<0.05 S				MZ $_{Subclinical}$ = 28.695 P<0.01 HS			

(*) **HS: Highly Sig. at P<0.01; S: Sig. at P<0.05**

Z - test based on difference between two proportions; Multiple Z - test based on difference among several proportions

Table & Figure (3) showed the distribution of mastitis infection according to quarter position which indicated that the infection significantly occur more in hindquarters than forequarters in both clinical and subclinical mastitis, these results were in agreement with **Saini et al. (1994)** and **Khan and Muhammad (2005)** who found that hindquarter prevalence was the highest (29%) , also with **Mustafa et al. (2011)** who found the same results (68.96%) in left hind and (19.54%) in right hind more than left and right fore quarters (0%) and (11.49%) respectively. Furthermore**,** we agreed with **Chavoshi and Husaini (2012)** who found (73%) hindquarters were mostly affected, and **Srinivasan et al. (2013)** who recorded the highest SCM occurrence was in hind quarters (83.34%) than fore quarters (16.36%). This may belong to the pendulous udder and long teat of buffaloes that may contributed to higher risk of mastitis **(Moroni et al. 2006, Fagiolo and Lai, 2007)**. Besides to the fact that those hind quarters always exposed to dung and urine, in addition to the stress on these quarters during the milking process **(Srinivasan et al. 2013)**.



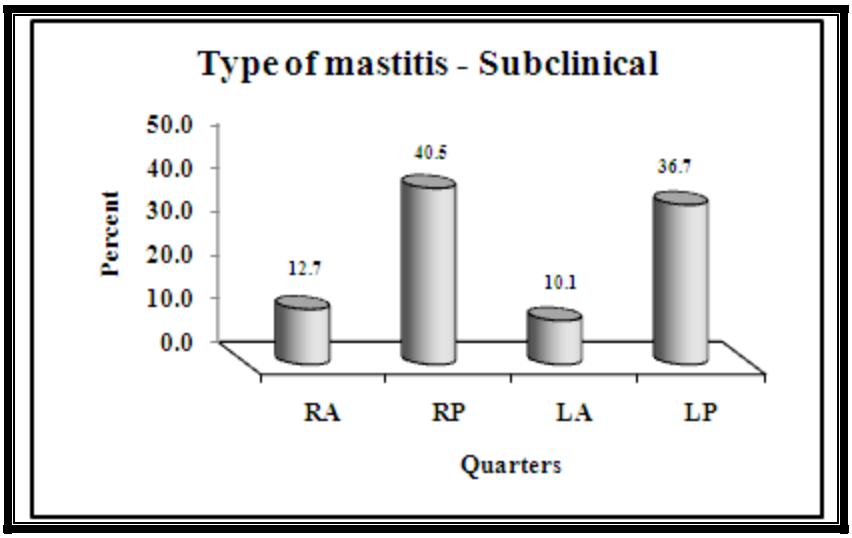


Figure (3): Frequency's percents of clinical and subclinical mastitis according to quarter position

However, buffalo's milk considered as an important source of market milk, It meets certain specific food requirements of human population **(Maniruzzaman et al. 2010).** But at the same time it is a perfect medium for the growth and multiplication of many kinds of microorganisms **(Harrigan et al. 1976; Soomro et al. 2002)**. These consequently lead to milk discoloration, putrefaction and rancidity defects in the milk , in addition to infectious diseases transmission to the consumers **(Maniruzzaman et al. 2010)** therefore , we worked in this study on bacterial isolation from milk samples too. In **Table (4)** we revealed the incidence of Staphylococcus spp. in clinical and subclinical quarters although they were non significant differences, we found that Staphylococcus chromogens 14(17.7%) was the predominant bacteria among Staphylococcus spp. followed by Staphylococcus aureus and Staphylococcus sciuri 5(6.3%) for each in subclinical infection while in clinical infection 2 isolates (14.3%) was detected as Staphylococcus sciuri.

Table (4) Incidence of Staphylococcus spp. bacteria in clinical and subclinical mastitis according to bacterial culture and VITEK identification technique

Staphylococcus spp.	No. of Clinical quarters	%	No. of Subclinical quarters	%
S. aureus	0	0.00	5	6.3
S. epidermidis	0	0.00	1	1.3
S. sciuri	2	14.3	5	6.3
S. lentus	0	0.00	4	5.1
S. chromogens	0	0.00	14	17.7
S. haemolyticus	0	0.00	3	3.8
S. xylosus	0	0.00	4	5.1
C.S (*) P-value.	K.S.=0.571 P>0.05 NS		K.S.=0.155 P>0.05 NS	

(*) NS: Sig. at P>0.05; K.S. - One-Sample Kolmogorov-Smirnov Test procedure compares the observed cumulative distribution function.
% are based on total no. of mastitis classified by Clinical and Subclinical.

Memon et al. (1999) found that Staphylococcus aureus was the dominant bacteria (38%) , **Bhalerao et al. (2000)** noted (54.55%) percent of Staphylococcus aureus in mastitis infection. Moreover, **Khan and Muhammad (2005)** reported a significant major pathogens in subclinical mastitis was Staphylococcus aureus 28(45%)**. Dhakal et al. (2007)** and **Baloch et al. (2011)** isolated the Staphylococcus aureus in ahighest rate (26.8%) , 34(48.57%) in

clinical infection respectively. **Abd El-Razik et al. (2010)** also isolated Staphylococcus aureus (15.62%) but as a second most pathogens in subclinical infection**. Srinivasan et al. (2013)** found Staphylococcus spp. was the most common pathogens isolated from SCM. **Aliaa et al. (2013)** also found Staphylococcus aureus was the highest isolate (13.1%). But we agreed in some degree with **Moroni et al. (2006)**, **Dhakal et al. (2007)** and **Vajdi Hokmabad et al. (2011)** who reported Coagulase Negative Staphylococci (CNSs) were the most common pathogens in mastitis cases.

Table (5) Incidence of Streptococcus spp. in clinical and subclinical mastitis according to bacterial culture and VITEK identification technique

Streptococcal spp.	No. of Clinical quarters	%	No. of Subclinical quarters	%
S. agalactiae	3	21.4	6	7.6
S. dysagalactiae	1	7.1	0	0.0
S. uberis	4	28.6	16	20.3
S. thoraltensis	0	0.0	3	3.8
S. canis	3	21.4	0	0.0
S. equi	1	7.1	1	1.3
E. faecium	0	0.0	2	2.5
E. gallinarum	0	0.0	3	3.8
E. casseliflavus	0	0.0	4	5.1
L. garvaiae	1	7.1	1	1.3
K. rosea	0	0.0	1	1.3
A. otitis	0	0.0	2	2.5
C.S (*) P-value.	K.S.=0.429 P<0.05 S		K.S.=0.314 P<0.01 HS	

(*) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05
K.S. - One-Sample Kolmogorov-Smirnov Test procedure compares the observed cumulative distribution function.
% Are based on total no. of mastitis classified by Clinical and Subclinical.

There were a significant differences of Steptococcus spp. in clinical and subclinical quarters showed in **Table (5)**, Streptococcus uberis represent the most common isolated bacteria 4(28.6%) and 16(20.3%) in both clinical and subclinical infection respectively , followed by Streptococcus agalactiae 3(21.4%) and 6(7.6%). **Khan and Muhammad (2005)** isolated (23%) Streptococcus agalactiae in their study to determine the quarter wise prevalence of mastitis in buffaloes and this represented higher rate than the rate presented in our study. **Moroni et al. (2006)** isolated Streptococcus spp. in (15%) persents in Italian buffaloes. **Abd El-Razik et al. (2010)** isolated Streptococcus agalactiae and Streptococcus dysagalactiae from subclinical infection (3.12%), (0.62%) respectively, and **Chavoshi and Husaini (2012)** isolated Streptococcus agalactiae from 3 quarters (8%) and this was close to our results in subclinical infection, also they isolated other Streptococcus spp. from 8 quarters (21%). **Srinivasan et al. (2013)** found Streptococcus spp. in 11 subclinical cases (20.37%) of the 54 bacterial isolates. While **Aliaa et al. (2013)** reported Streptococcus agalactiae (4.4%) Streptococcus dysagalactiae (5.6%).and we disagreed with **Baloch et al. (2011)** who isolated Streptococcus uberis in only (4.28%) and Streptococcus dysagalactiae in (11.42%) in clinical mastitis. However, it is very important to mention that we isolated some species of Streptococcus bacteria in this study that we did not find any authors presented such findings in buffalo mastitis, such as Streptococcus canis from clinical infected quarters 3(21.4%) , Streptococcus equi 1(7.1%) from clinical quarters and 1(1.35) from subclinical quarters , these may attributed to the presence of other animals reared in same place with buffaloes that contaminated the surrounding areas of buffaloes , like horses and dogs these usually reared by farmers as a carrying animals and for guarding respectively.

Table (6) Incidence of gram negative bacteria in clinical and subclinical mastitis according to bacterial culture and VITEK identification technique

Gram negative bacteria	No. of Clinical quarters	%	No. of Subclinical quarters	%
Enterobacteriacae G-ve *	0	0.0	13	16.5
Other G-ve **	2	14.3	1	1.30
C.S (*) P-value.	P=.500 NS		P=0.001 HS	

(*) NS: Sig. at P>0.05

Binomial Test procedure compares the observed frequencies of the two categories of a dichotomous variable.

% Are based on total no. of mastitis classified by Clinical and Subclinical.

***Enterobacteriacae G-ve refers to (E.coli, Klebsiella spp.)**

****Other G-ve refers to (Aeromonas spp.)**

Furthermore, gram negative bacterial species recorded in **Table (6**), represented by two group Enterobacteriacae spp. these isolated from 13 subclinical quarters (16.5%) and other gram negative bacteria isolated from 2 clinical quarter (14.3%), and only 1 subclinical quarter (1.30%) a significant differences showed in subclinical quarters but in clinical quarters the occurrence of other gram negative bacteria was not significant. according to this results we found that Enterobacteriacae spp. occured entirely in subclinical mastitis while the other gram negative species represented by Aeromonas spp. occured in low rate in both clinical and subclinical quarters, we should noted that no study isolated other gram negative bacteria that refers to Aeromonas spp. from cases of buffalo mastitis.

Khan and Muhammad (2005) isolated E.coli from 11cases (18%) of buffaloes mastitis this was highest than our rate of Enterobacteriacae spp. isolation from 13 subclinical quarters (16.5%). **Dhakal et al. (2007)** reported E.coli (7.1%) in clinical infection this was opposite to our results because we did not isolated the Enterobacteriacae spp. from clinical quarters, also he found (2.8%) E.coli in subclinical infection, Klebsiella pneumonia (3.6%) and these species considered as members in Enterobacteriacae family and the summation of their percentage was (6.4%) although that it was lower than our percentage in subclinical quarters of (16.5%) for Enterobacteriacae spp. also **Baloch et al. (2011)** revealed E.coli from 7 samples (10%) which represented another non similarity between his results and ours. Nevertheless, **Vajdi Hokmabad et al. (2011)** found that Coliforms were not probably very important in buffalo's intramammary infections. **Srinivasan et al. (2013)** isolated E.coli in 6 subclinical cases (11.11%).But we agreed with **Abd El-Razik et al. (2010)** who found that the incidence of subclinical mastitis in buffaloes depending on the bacterial cultivation was the highest in E.coli (23.75%) and **Aliaa et al. (2013)** isolated E.coli infection (13.9%) and Klebsiella spp. (2.2%) in subclinical infection in Egyptian dairy buffaloes these results were somewhat resemble to our recovered rate of Enterobacteriacae spp. from 13 subclinical quarters (16.5%). After all, the presence of Enterobacteriacae spp. in infected quarters indicated the contamination of udder as well as environment of the animals with fecal materials and manure which found an opportunities for entering the teat orifice, reaching to udder tissue and started the inflammatory process of mastitis. Or it may belong to indiscriminative and intensive uses of antibiotics targeted against gram positive microbes **(Radostitis et al. 2000)**.

We concluded that mastitis in buffaloes in our country formed an important dairy disease and must have more attention in order to provide enough information about its occurrence that help in putting a suitable plans to control this unobvious disease, and due to importance of gram negative pathogens of mastitis we need more studies to determine the exact species that responsible for infections in these animals, in addition to increase research area to cover all Baghdad's lands in which buffaloes were reared.

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