PREVALENCE, ETIOLOGY AND ANTIBIOGRAM OF MASTITIS IN BUFFALOES IN SOHAG GOVERNORATE

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ABSTRACT

In this study total of 416 milk samples (104 buffaloes) which were reared in a smallholder private cases hand milked in Sohag Governorate were examined. Clinical screening of examined animals beside the results of California mastitis test for apparently healthy animals revealed that the overall prevalence rate of clinical and subclinical mastitis were 22.1% and 26.9%, respectively. The incidence of one quarter infection was the highest followed by all quarters in clinical and subclinical mastitis cases at the percentage of 30.4%, 35.7%, 26.2% and 28.6% respectively. The third lactating season was the highest incidence of both clinical and subclinical mastitis at the percentage of 28.6% and 35.3% respectively. By bacteriological examination 56 isolates from clinical mastitic cases and 68 isolates from subclinical mastitic animals were detected. *Staphylococcus aureus , E.coli* and *, Streptococcus agalactia* were isolated by ratio 16.1%, 33.9%, 14.3% and 29.4%, 22.1%,10.3 in cases of clinical mastitis and subclincal ones respectively. *staphylococcus aureus* and *E. coli staphylococcus aureus and streptococcus agalactia* and *staphylococcus aureus* and *E-coli* microorganisms were mixed isolated by ratio 7.1%, 7.1% and 5.4% in cases of clinical mastitis and 8.8%,4.4%, 4.4% in subclinical mastitic cases. Mixing of more than 3 isolates or more was the rate of clinical mastitis 8.9%, whereas subclinical mastitic were 8.8%. Simplex and multiplex PCR assays showed that *E-coli* isolates were the highest causative agents of clinical mastitis and *staphylococcus aureus* was the major cause of subclinical mastitic ones. Antimicrobial susceptibility testing revealed that all isolates were highly sensitive to Gentamycin Ceprofloxacin, Lincomycin and Novobiocin followed by Chloramphenicol, Cephalothin and Amoxycillin.

Key words: Buffalo, lactation, clinical & subclinical mastitis, prevalence.

INTRODUCTION

Mastitis is still the most important and widespread zoonotic production disease in milking herds in all species of farm animals, despite several decades of research and remedial efforts. It has a long range of damaging effects on productive performance of animals (Seegers *et al.,* 2003).

In Egypt buffalo appears to be the first animal for milk yield rather than meat production and approx. 65% (or may be more) of the daily milk production in Egypt is obtained from dairy buffaloes (Metry, 1996). The majority of Egyptian buffaloes' population is apparently still under the farmer's hand (private cases) rather than that those kept on farms for milk and meat production. However, the majority of literature on mastitis of dairy animals at Upper Egypt is focused on Friesian cows and on buffaloes that located on farms (Seddek *et al.,* 1999).

Clinical and subclinical mastitis recorded in buffalo and it considered one of the most economically important deadly diseases of milky animals, and causes the changes in glandular tissues affecting quality and quantity of the milk (Sharma and Sindhu, 2007). Mastitis is characterized by physical, chemical, bacteriological, cytological changes in milk and pathological changes in the gland. Clinical mastitis recognized by abnormal milk, gland swelling and /or systemic illness whereas subclinical mastitis characterized by apparently normal milk with an increase in SCC due to influx of leukocyte with reduces in milk production. (Philip *et al.,* 1993).

Mastitis is defined as an inflammatory reaction of the parenchyma of the mammary gland that can be of an infectious, traumatic or toxic nature (International Dairy Federation 1987). Mastitis may result in the presence of bacteria and other infectious agents which may be harmful to humans, (Reyher and Dohoo, 2011). The prevalence of intramammary infection in buffalo was 66%. Since the mammary gland is highly susceptible to infection during the periparturient period, the incidence was highest during the 30 days

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after calving. Since mastitis is a diseases caused by multiple factors (multiple pathogens), it is difficult to control (Andrei *et al.,* 2011).

Over one hundred different microorganisms have been isolated from bovine mastitis, but the most frequently isolated microorganisms are Staphylococci, Streptococci and Gram-negative bacteria *(Hussain et al.,* 2012; Hussain *et al.,* 2013). Mastitis-causing bacteria can be classified in contagious as Streptococcus *agalactiae, Stapylococcus aureus, Arcanobacter piogenes*, *Micoplasm*a; environmental as *Streptococcus uberis, and dysgalactiae, Escherichia coli*, *Enterobacteriacee,* yeasts and moulds (*Prototheca zoophii)* and opportunist as *coagulase negative Staphilococcus C.N.S* (Galiero, 2002). In buffalo Incidence of subclinical mastitis more prevalent than clinical mastitis in housed buffaloes in percentages 18.5% and 9% respectively. *S. aureus, E. coli, St agalactia and St. dysgalactia* were the most common isolates in clinical mastitis. *E. coli, S. aureus, C.N.S, Pseudomonas, St agalactia,* and *St. dysgalactia* were the most common isolates in subclinical mastitis. Mixed infection by *S. aureus and E. coli* common cause in clinical mastitis 24.4% and *S. aureus* and *C.N.S* common cause in subclinical mastitis 18.9% (Abd-Elrahman, 2013).

Due to the limitations of cultural methods, the development of PCR-based methods provides a promising option for the rapid identification of bacteria. With these methods, identification of bacterial pathogens can be made in hours, rather than the days required for conventional cultural methods Yu-Ping *et al.* (2007). PCR can also improve the level of detection due to its high sensitivity. Theoretically, only a few cells of the pathogen are necessary to yield a positive diagnosis The presence of pathogens may be detected at earlier stages of infection and in carrier animals, when the numbers of bacteria in milk may be very low (Pradhan *et al.,* 2011).

The present study was designed to see the prevalence of mastitis (clinical and subclinical) in buffaloes in relation to lactation season, quarters affected, injury on the teat, involvement of different organisms and their antimicrobial sensitivity. Also it is aimed to use PCR assays for the detection of *Staphylococcus aureus*, *Escherichia coli and Streptococcus agalactiae* in mastitic milk samples.

MATERIALS AND METHODS

Animals

Sample collection:

416 quarter milk samples (104 buffaloes) were collected from small holder dairy farms at age of 3-6 years and 1-4 lactating seasons from September till December 2018 at Sohag Governorate. Signs and symptoms were recorded to clinical mastitic cases (Schalm *et al.,* 1976). Quarter milk samples from udder were categorized as subclinical mastitis based on the following criteria: absences of visible abnormalities of milk secretions, California mastitis test (CMT) (Gonzalez *et al.,* 1990).

California Mastitis Test (CMT)*:*
The CMT was used alongside the physical examinations and the test was carried out as described. Equal volume of milk samples which were taken during milking time and the CMT reagent (2ml of each quarter) and was mixed thoroughly in a cup of black plastic paddle; the mixture was gently rotated for 10 seconds, and then results were recorded (Moroni *et al.,* 2006).

Estimation of Somatic Cell Count (SCC):
Milk samples were thoroughly mixed and from each sample 0.01 ml was spread over an area measures 1 cm² of a glass slide then left to air dried and stained by Newman-Lampert stain and examined microscopically according to (Alekish *et al.,* 2014).

Milk sampling
The infected teat end was disinfected with cotton soaked in 70% ethyl alcohol. The first few streams of foremilk were discarded. Samples for bacteriological analysis were then collected into screw capped sterile McCartney bottle and were held on ice until delivery to the laboratory within 20 to 30 min of collection the samples were preserved at $-20 \, C^o$ till send to Animal Health Research Institute for Bacterial isolation and identification. (Soomro *et al.,* 2002).

Bacteriological examinations:
Bacteriological isolation and identification was done on specific media for *S.aureus* (sheep blood agar, manitol agar and brain heart infusion agar), specific media for *Strept. agalacia* (Edward media), and specific media for *E-coli* and *coliforms* (MacConky agar) according to (Toply and Welson 1998). Identification of the isolated organisms was done according to (APHA 1992, Koneman *et al.,* 1992, Collee *et al.,* 1996 and Quinn *et al.,* 2002) based on their colony morphology, hemolytic pattern on blood agar, microscopic examination (Gram staining) and biochemically for glucose, sucrose and maltose fermentation, catalase activity, gelatine liquefaction, urea production, methyl red and nitrate reduction were adopted.

Antimicrobial sensitivity test :
All the bacteria isolated through microbiological procedures were subjected to antimicrobial susceptibility testing by disc diffusion method (Anonymous, 2004). The sensitivity against Gentamycin10Ug, Ceprofloxacin 5Ug, Chloramphenicol 30Ug, Cephalothin 30Ug, Sulph-trim10Ug, Amoxycillin 25Ug, Lincomycin 10Ug,

Oxytetracycline 30Ug, Novobiocin 10Ug, Erythromycin 5Ug, Ampiclox30Ug and Pencillin G30Ugwas determined on Muellar-Hinton agar as described by National Committee for Clinical Laboratory Standards

DNA Extraction:

Milk samples were mixed and a 300-µl sample added to 300 µl of lysis buffer (0.1M NaCl, 20 mM Tris-HCl, 1mM EDTA, 0.5% SDS and 100 µg of proteinase K/ml). After incubation at 37°C for 4 h. DNA was extracted and purified using phenol-chloroform method as described previously by (Sambrook *et al.,* 1989). The dried DNA pellet was dissolved in 50 µl of TE buffer (10 mM Tris HCl -1 mM EDTA, pH 7.8) and stored at -20°C until used.

Oligonucleotide Primers:

Primers were synthesized, by (Fermentas, AB.Gene), (MWG, oligosynthesis - Germany). The sequences of different primers (Riffon *et al.,* 2001) included *Staphylococcus aureus* specific primer (Forward: 5` GGA CGA CAT TAG ACG AAT CA 3` and reverse: 5` CGG GCA CCT ATT TTC TAT CT 3`); *Escherichia coli* specific primer (Forward: 5` ATC AAC CGA GAT TCCAGT 3` and reverse: 5` TCA CTA TCG GTC AGT CAG GAG 3`) and *Streptococcus agalactiae* specific primer (Forward: 5` CGC TGAGGT TTG GTG TTT ACA 3` and reverse: 5` CAC TCC TAC CAA CGT TCT TC 3`).

PCR Assays:

Simplex PCR assay for amplification of each organism was performed using 25 µl PCR reaction volume. 12.5 µl of 2X PCR master mix (Fermentas, Germany), 150 ng of the DNA template, 0.5 µM of each primer and Up to 25 µl Nuclease free water were mixed in a PCR tube. The amplification was performed in a programmable heating block, (Primus Thermal Cycler, MWG Biotech, Germany). A total of 35 PCR cycles were run under the following

conditions; denaturation at 94°C for 45 sec, annealing for 1 min (at 64 and 60°C for *Staphylococcus aureus* & *Escherichia coli* and *Streptococcus agalactiae*, respectively) and extension at 72°C for 2 min. After final cycle the preparations were kept for 10 min at 72°C to complete the reaction.

A one step multiplex PCR was developed using each of the primer sets previously used for the simplex PCR. 25 µl PCR reaction volume containing 12.5 µl of 2X PCR master mix (Fermentas, Germany), 0.5 mM $MgCl_2$, 250 ng of the DNA template, 25 pmol of each primer and Up to 25 µl Nuclease free water were mixed in a PCR tube. A total of 40 PCR cycles were run under the following conditions; denaturation at 94°C for 45 sec, annealing for 1 min at 62°C and extension at 72°C for 2 min. After final cycle the preparations were kept for 10 min at 72°C.

Amplification products were electrophoresed in 1.7% agarose gel containing 0.5X TBE at 70 volts for 60 min. and visualized under ultraviolet light. To assure that the amplification products were of the expected size, a 100 bp DNA ladder was run simultaneously as a marker. Presence of 1318 bp, 232 bp and 405 bp DNA fragments indicated the presence of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus agalactiae* DNA, respectively.

Statistical analysis

The cumulative data was entered in Microsoft Excel for analysis of the data, and $P<0.05$ was regarded as significant. Incidence risks of clinical mastitis were computed by dividing the number of occurrences of clinical mastitis during a defined period by average number of lactating cows during that period. The seasonal variation of clinical mastitis incidence was tested with Edward's test corrected for changing size of the population at risk in different months Norusis M J (1993).

RESULTS

Table 1: Clinical examination of udder and milk secretion in healthy and diseased buffaloes.

	Udder	Milk (by necked eye)	
Size	81 animals had symmetrical udder– 23 asymmetrical	Normal milk	104
shape	81 normal key shape- 10 abdominal- 7 round- 6 stepped	Milk with watery appearance	8
Teat tip	89 normal round -15 pointed	Milk with bloody appearance	5
Skin	81normal pale red -23 hyperemic	Milk with mucous	6
Surrounding tissue	94 normal-10 with scars	Milk with flakes	4

Table 2: Incidence of clinical and subclinical mastitis in examined buffaloes.

	No. of affected animals	%	No. of examined quarters	%	No. of affected quarters	%
Clinical mastitis	23	22.1	92	22.1	56	13.4
Sub clinical mastitis	28	26.9	112	26.9	68	16.3
Healthy	53	51.0	212	51.0	292	70.3
total	104	100	416	100	416	100

Table 3: The distribution of infected quarters in the clinically and subclinically mastitic buffaloes.

Lactation Season	No. of animals at risk	Clinical mastitis		Sub clinical mastitis	
		No. of diseased animals	%	No. of diseased animals No.	%
1	20	3	15.0	4	20.0
2	27	4	14.8	5	18.5
3	35	10	28.6	12	35.3
4	22	6	27.3	7	31.8
Total of diseased animals	104	23	22.1	28	26.9

*1st lactation season means a buffalo has 3 years old-   *2nd lactation season means a buffalo has 4 years old
*3rd lactation season means a buffalo has 5 years old   -   *4th lactation season means a buffalo has 6 years

Table 4: The effect of lactation season on mastitis incidence in examined buffaloes.

No of animals Studied	Type of Mastitis	Total affected animals	One quarter		two quarters		Three quarters		Four quarters
			No.	%	No.	%	No.	%	%
104	Clinical	23	7	30.4	5	21.7	5	21.7	6	26.2
	Sub clinical	28	10	35.7	4	14.3	6	21.4	8	28.6

Table 5: Detection of S. aureus, E. coli and St. agalactiae in 124 milk samples by culture, simplex PCR and multiplex PCR Test method

Bacteria isolated	Culture				Simplex PCR				Multiplex PCR			
	Clinical mastitis		Sub-clinical mastitis		Clinical mastitis		Sub-clinical mastitis		Clinical mastitis		Sub-clinical mastitis	
Bacteria	N	%	N	%	N	%	N	%	N	%	N	%
S. aureus	9	16.1	20	29.4	10	17.9	23	33.8	10	17.9	21	30.9
E. coli	19	33.9	15	22.1	20	35.7	17	25.0	19	33.9	16	23.5
St. agalactiae	8	14.3	7	10.3	9	16.1	8	11.8	9	16.1	8	11.8
S. aureus+ E. coli	4	7.1	6	8.8	4	7.1	6	8.8	4	7.1	6	8.8
S. aureus+ St.agalactiae	4	7.1	3	4.4	4	7.1	3	4.4	4	7.1	4	5.9
St. agalactiae+ E. coli	3	5.4	3	4.4	3	5.4	3	4.4	3	5.4	3	4.4
other bacteria	4	7.1	8	11.8	1	1.8	2	2.9	2	3.6	5	7.4
Mixed 3 or more bacteria	5	8.9	6	8.8	5	8.9	6	8.8	5	8.9	5	7.4
Total	56	-	68	-	56	-	68	-	56	-	68	-

Table 6: In vitro sensitivity of different microorganisms to different antibiotics

Antibiotics		Staphylococcus aureus(No:46)	Streptococcus agalactiae(No:28)	E. coli (No:50)
Gentamycin	10Ug	+++	+++	+++
Ceprofloxacin	5Ug	+++	+++	+++
Chloramphenicol	30Ug	++	+++	+++
Cephalothin	30Ug	++	+++	++
Sulph-trim.	10Ug	++	++	+++
Amoxycillin	25Ug	++	++	+++
Lincomycin	10Ug	+++	+	++
Oxytetracycline	30Ug	++	+++	++
Novobiocin	10Ug	+++	++	++
Erythromycin	5Ug	++	++	+
Ampiclox	30Ug	+	++	+
Pencillin G	30Ug	+	+++	+

Table 7: Correlation between SCC / ml. and single bacterial infection in the examined milk sample.

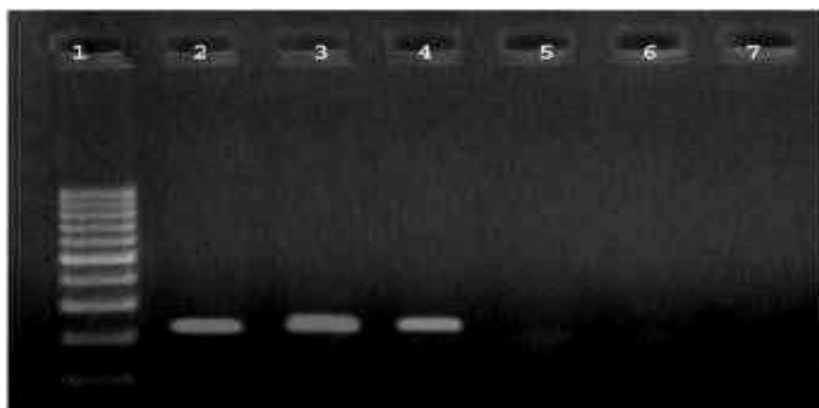| Bacterial infection	Minimum SCC / ml	Maximum SCC / ml	Mean	±SEM
Staphylococcus aureus	13000	200000	28420.6897	8835.53810
Escherichia coli	320000	320000	102805.8824	16195.05324
Streptococcus agalactiae	13000	17000	14500	284.52132

Fig. 1: Polymerase chain reaction products (1318 bp) amplified from Staphylococcus aureus DNA. Lane 3, positive clinical mastitic milk sample; Lane 4, positive sub-clinical mastitic milk sample. Lane 5, negative clinical mastitic milk sample; Lane 6, negative sub-clinical mastitic milk sample. Lane 2, Control positive; Lane 7, Control negative and Lane 1, 100 bp DNA marker

Fig. 2: PCR products (232 bp) amplified from Escherichia coli DNA. Lane 3, positive clinical mastitic milk sample; Lane 4, positive sub-clinical mastitic milk sample. Lane 5, negative clinical mastitic milk sample; Lane 6, negative sub-clinical mastitic milk sample. Lane 2, Control positive; Lane 7, Negative control and Lane 1, 100 bp DNA marker.

Fig. 3: PCR products (405 bp) amplified from Streptococcus agalactiae DNA from: Lane 3, positive clinical mastitic milk sample; Lane 4, positive sub-clinical mastitic milk sample. Lane 5, negative clinical mastitic milk sample; Lane 6, negative sub-clinical mastitic milk sample. Lane 2, Control positive; Lane 7, Negative control and Lane 1,100 bp DNA marker

Fig. 4: Multiplex PCR products amplified from DNA. Lane 2, Staphylococcus aureus; Lane 3, Streptococcus agalactiae; Lane 4, Escherichia coli; Lane 5, Staphylococcus aureus and Escherichia coli; Lane 6, Staphylococcus aureus and Streptococcus agalactiae; Lane 7, Streptococcus agalactiae and Escherichia coli. Lane 1, 100 bp DNA marker and Lane 8, Negative control.

DISCUSSION

Mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses (Sharma *et al.,* 2007).

In the present study table (1) results revealed that by inspection and palpation the udder of examined animals were asymmetrical size in 23 cases, the tip were pointed in 15 cases, the skin were hyperemic in 23 cases and 10 animals had scars on surrounding tissue. This agreed with (Yasser *et al.,* 2013) who suggested that abnormal udder shape could be considered as predisposing factor to mastitis. Examination of milk by necked eye showed that 4 cases had flakes, milk with bloody appearance were seen in 5 cases and milk with mucous were seen in 6 cases. In mastitis, there is drastic change in the milk,

taste and consistency. Muhammad *et al.* (2005) also recorded that the changes in the milk due to mastitis are shown as milk with bad taste and odor was found 8.08% in buffalo and 6.74% in cow. Milk mixed with mucus and blood was recorded 6.61% and 7.35% in buffalo and in cow 7.35% and 5.61% while milk with mixed mucus and blood was 5.51% in buffalo and 4.49% in cow, respectively.

The results obtained in table (2) revealed that from 104 animals examined the prevalence of clinical mastitis in buffaloes were recorded in 23 animals by ratio of (22.1%). These findings are in close alignment with the findings of (Bilal *et al.,* 2004). The prevalence of sub-clinical mastitis was also found higher in buffaloes that reported in 28 animals by ratio of 26.9% which agree with (Dangore *et al.,* 2000).

There was a large variation in the prevalence of clinical and subclinical mastitis rates which may be

attributed to some mangemental factors such as using of dry buffaloes therapy, feeding patterns, heifer replacement rates, environmental condition surroundings the animals, prevalent microorganism (McDougall *et al.,* 2014).

Table (3) the higher incidence of clinical mastitis and subclinical mastitis in one quarters then four quarters by ratio of 30.4%, 35.7%, 26.2% and 28.6% respectively were recorded as also by (Didonet *et al.,* 1986 and Karimuribo *et al.,* 2006). Nakov and Trajcev (2012) also reported that in the most cases of clinical mastitis there was affected only one quarter of the mammary gland (74.35%), two quarters in 20.13%, three quarters to 3.61% and four quarters were affected in 1.89% of the cases of clinical mastitis.

As shown in table *(*4) the ratio of the clinical mastitis was 15% in the 1st lactation season of the examined animals, 14.8% in the 2nd lactation season, 28.6% in the 3rd lactation season and 27.3% in the 4th lactation season while the percent of sub clinical mastitis was 20.0%, 18.5%, 35.3% and 31.8% in the 1st, 2nd, 3rd, and 4th lactation season respectively, the result obtained are going hand by hand with (Pal and Verma 1988 and Alam *et al., 2004*) who reported the highest incidence at 3rd calving, and agree with *(*Kavitha *et al.,* 2009 and Sharma *et al., 2004)* who found that, As the parity increases, an increase in the incidence of mastitis is seen (also found higher prevalence of subclinical mastitis in 5 to 9 years old animals and in 3rd and 4th parities. The difference found among reports may be due to various factors such as breed, season, and husbandry system (Moroni *et al., 2006)*.

Most of the mastitis cases are of infectious etiology (Deb *et al.* 2013). Table *(*5) Out of the 56 milk samples collected from clinical cases and examined bacteriologically; *Staphylococcus aureus, Escherichia coli and Streptococcus agalactiae* were recovered and identified in percentages of 16.1%, 33.9% and 14.3%, respectively. Dual bacterial isolation [*Staphylococcus aureus* and *Escherichia coli* (7.1%); *Staphylococcus aureus* and *Streptococcus agalactiae* (7.1%); *Streptococcus agalactia*e and *Escherichia coli* (5.4%)] was also detected. Negative samples revealed 7.1% and 8.9% revealed mixed cultures of three or more environmental bacteria and accordingly were considered contaminated. On the other hand, the 68 milk samples from sub-clinical cases were examined bacteriologically. Bacterial isolates were identified as *Staphylococcus aureus* (29.4%), *Escherichia coli* (22.1%) and *Streptococcus agalactiae* (10.3%). Dual bacterial isolation [*Staphylococcus aureus* and *Escherichia coli* (8.8%); *Staphylococcus aureus* and *Streptococcus agalactiae* (4.4%); and *Streptococcus agalactiae* and *Escherichia coli* (4.4%)] was also detected. Also, 11.8 and 8.8% of samples revealed negative and mixed cultures of three or more environmental

bacteria, respectively. The results presented here are similar to previous studies by (Döpfer *et al.,*1999 and Su, *et al.,*2000).

The number of mastitic milk samples positive for selected major pathogens of mastitis in the individual simplex PCR assays is listed in table 5. The reactivity of used primer sets with *Staphylococcus aureus, Escherichia coli* and *Streptococcus agalactiae* in mastitic milk samples is shown in Figures 1, 2 and 3, respectively. The results indicated that, out of 56 milk samples from clinical cases of mastitis, 17.9, 35.7 and 16.1% of the samples were reacted positive when tested using *Staphylococcus aureus, Escherichia coli* and *Streptococcus agalactiae* specific primer sets, respectively. On the other hand, out of 68 milk samples from sub -clinical cases of mastitis, 33.8%, 25.0% and 11.8% of the samples were reacted positive when tested using *Staphylococcus aureus, Escherichia coli* and *Streptococcus agalactiae* specific primer sets, respectively. Dual bacterial detection [*Staphylococcus aureus* and *Escherichia coli* (7.1%); *Staphylococcus aureus* and *Streptococcus agalactiae* (7.1%); and *Streptococcus agalactiae* and *Escherichia coli* (5.4%)] were also indicated in samples from clinical mastitic cows and 8.8, 4.4 and 4.4%, respectively, in samples from sub-clinical mastitic cows. Also, 1.8 and 2.9% samples were negative in samples from clinical and sub-clinical mastitic cows, respectively which agree with (Zadoks and Schukken 2006).

The data in table 5 and Figure 4 also showed the results of multiplex PCR using different primer sets of *Staphylococcus aureus, Escherichia coli* and *Streptococcus agalactiae*. Out of 56 milk samples from clinical cases of mastitis, 17.9, 33.9 and 16.1% of the samples were reacted positive, respectively. On the other hand, out of 68 milk samples from sub-clinical cases of mastitis, 30.9, 23.5 and 11.8% of the samples were reacted positive, respectively. Dual bacterial detection [*Staphylococcus aureus* and *E. coli* (7.1%); *Staphylococcus aureus* and *Streptococcus agalactiae* (7.1%); and *Streptococcus agalactiae* and *Escherichia coli* (5.4%)] was also indicated in samples from clinical mastitic cows and 8.8, 5.9 and 4.4%, respectively, in samples from sub-clinical mastitic cows. Also, 3.6 and 7.4% samples revealed negative in samples from clinical and sub-clinical mastitic cows, respectively. Phuektes *et al*. (2003) reported the same results. The obtained result are parallel to result obtained by (Abd-Elrahman 2013), who found that *S. aureus, E. coli, St agalactia* and *St. dysgalactia* were the most common isolates in clinical mastitis. *E. coli, S. aureus, C.N.S, Pseudomonas, St agalactia*, and *St. dysgalactia* were the most common isolates in subclinical mastitis.

In table (6) Antibiogram profile using 12 different antibiotics revealed that the most isolates of *Staphylococcus aureus* were highly sensitive to gentamycin cebrofloxacin, lincomycin, and

novobiocin That similar results were obtained by (İkiz *et al.,* 2013). These organisms showed less sensitivity to Chloramphenicol, Cephalothin, Sulph-trim., Amoxycillin, Oxytetracycline, Erythromycin, Ampiclox and Pencillin G. Abdel – Khalek and El-Sherbini (2005) their results suggest that Penicillin is no longer the antimicrobial of choice for staphylococcal mastitis. Sensitivity of *Streptococcus spp*. To gentamycin cebrofloxacin, chloramphenicol, cephalothin, oxytetracycline and penicillin G were very high in this study but Sulph-trim, Amoxycillin, Lincomycin Novobiocin, Erythromycin and Ampiclox were less sensitive (Abd El-Hafeez, 2002). *E.coli* were highly sensitive to gentamycin, ciprofloxacin, chloramphenicol, sulfa-trim and amoxycilline. The obtained results were agreed with (CLSI. 2008). But Cephalothin, Lincomycin, Oxytetracycline, Novobiocin, Erythromycin, Ampiclox and Pencillin G were less sensitive as shown in (Table 5). Rehman (1995) and Rocha *et al.* (2014) also observed same sensitivity of *E.coli.*

In table (7) the inflammatory reaction caused by intra-mammary infection is most commonly measured as an increase in SCC. The samples which contained single infection with *Escherichia coli* recorded the highest mean SCC (102805.8824±16195.05324), in case of *Streptococcus agalactiae*, the samples recorded the lowest mean SCC (14500±284.52132), while the samples containing *Staphylococcus aureus* recorded an intermediate mean SCC (28420.6897±8835.5381). These results substantiate with (Middleton *et al.,* 2004) and disagree with (De Haas *et al.,* 2004) who noted that clinical E. coli mastitis was significantly associated with the presence of a short peak in SCC, whereas Staph. aureus was associated with long increased SCC. Streptococcus dysgalactiae was not strongly associated with any of the defined patterns of peaks in SCC.

CONCLUSION

The dairy livestock of Sohag governorate were suffering a large prevalence of clinical and subclinical mastitis which unnoticed and very fewer farmers were aware of it. The *staphyloccal* mastitis was predominant for the infectious cause followed by *coliform* and *streptococcal* spp. From this study, it is concluded that the Gentamycin Ceprofloxacin, Lincomycin and Novobiocin are the most sensitive chemotherapeutic agents where the dairy animal management was poor. Due to limitation of cultural methods, PCR assays can be used as a rapid, sensitive and specific routinely diagnostic tool to detect the presence of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus agalactiae* in milk samples.

RECOMMENDATIONS

1) Health care of the teats beside the cleanliness of animal pens.

2) Treatment of cases of clinical mastitis.
3) Treatment of animals during the period of dryness (55 - 60 days before birth).
4) Elimination of cattle infections chronic fever.
5) it is mandatory that antibiogram study is made from time to time in a locality to be on the lookout of the most effective drug against the prevailing mastitogens i.e., bacteria.
6) Treatment of cases of hoof infection, especially in cattle public. Which can lead to inflammation in the udder or uterus.
7) The farmers should be inspired for mastitis management, udder health management, shed management and nutrition management through training, workshops, tours, farmers' schools for hygienic milk production in commercial scale

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انتشار واسباب والمضادات الحيويه لالتهاب الضرع فى الجاموس فى محافظه سوهاج

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فى هذه الدراسة تم فحص ٤١٦ عينه لبن من عدد ١٠٤ جاموس جميعها ملكية خاصة وفى مجموعات صغيرة وتحلب يدويا بمحافظة سوهاج. اتضح من الفحص الاكلينيكى لضروع الحيوانات المصابة ظاهريا مع نتائج اختيار كاليفورنيا للحيوانات السليمه ظاهريا ان معدل الانتشار العام لمرضى التهاب الضرع الظاهرى والخفى كان ٢٢.١% و ٢٦.٩% على التوالى. وكانت نسبة اصابة ضرع واحد هى الاعلى تليها الاصابة بكل الارباع بكل من حالات الالتهاب الظاهرى والخفى بنسب ٣٠.٤%, ٣٥.٧%, ٢٦.٢% و ٢٨.٦% على التوالى. وكان موسم الارضاع الثالث هو الاعلى نسبة اصابة بكل من حالات الالتهاب الظاهرى والخفى بسبة ٢٨.٦% و ٣٥.٣% بالترتيب. وبالفحص البكتريولوجى تم عزل ٥٦ عترة من الحالات المصابة بالتهاب الضرع الظاهرى و٦٨ عترة من المصابة بالتهاب الضرع الخفى على النحو التالى : تم عزل الميكروب العنقودى الذهبى والميكروب القولونى والمكور السبحى اجالاكتيا كل منفرد بنسب ١٦.١ % , ٣٣.٩ % و ١٤.٣ % بحالات الاتهاب الظاهرى وكانت النسب ٢٩.٤%, ٢٢.١% و١٠.٣% بحالات الالتهاب الخفى. وتم عزل كل من الميكروب العنقودى الذهبى والميكروب القولونى مختلطين وايضا الميكروب العنقودى الذهبى والمكور السبحى اجالاكتيا وايضا المكور السبحى اجالاكتيا والميكروب القولونى بنسب ٧.١%, ٧.١% و ٥.٤ % بحالات بالتهاب الضرع الظاهرى اما الخفى فكانت النسب عالتوالى ٨.٨%, ٤.٤% و ٤.٤%. اما اختلاط اكثر من ٣ ميكوبات او اكثر فكانت النسبة بالتهاب الضرع الظاهرى ٨.٩% اما الخفى ٨.٨%. باستخدام المقايسات البسيط والمتعدد لتفاعل البلمرة المتسلسل اثبت ان ميكروب القولونى هو الاعلى مسبب للاصابة بالتهاب الضرع الظاهرى وبينما كان ميكروب المكور العنقودى الذهبى الاعلى مسبب للاصابة بالتهاب الضرع الخفى . وباجراء اختبار الحساسية لاهم هذه العترات كل على حده ضد ١٢ نوع من المضادات الحيويه المختلفة اسفرت النتائج عن حساسيه العترات للجينتاميسن , سيبروفلوكساسين , لينكوميسين و نوفوبيسين يليها كلورمفينيكول , سيفالوسين وامكسيسسيللين.