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MOLECULAR STUDY OF SOME VIRULENCE GENES OF ENVIRONMENTAL STRPTOCOCCI ISOLATED FROM BOVINE SUBCLINICAL MASTITIS IN KAFR ELSHEIKH GOVERNORATE

By

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ABSTRACT

The aim of this study was to investigate the genotypic characteristics of environmental Streptococci *(Streptococcus uberis and Streptococcus dysgalactiae) isolated* from subclinical mastitis (SCM) cases, and to examine the possible association of some virulence genes between both species. Samples collected from 4 dairy farms, (653) quarter milk samples / 169 animals from 2 dairy cow's farms and (531) quarter milk samples / 135 animals from 2 dairy buffalo's farms were examined by California mastitis test (CMT). The results of CMT scores 1, 2 and 3 positives were 373 quarters (57.1%) divided into 75 (20.1%), 122 (32.7%) & 176 (47.2%) in cow's dairy farms. On the other hand, the results of CMT scores 1, 2 and 3 positives were 219 quarters (41.2%) divided into 94 (42.9%), 81 (37.0%) & 44 (20.1%) respectively in buffalo's dairy farms. A total of 79 (21.2 %) & 33 (15.1 %) environmental *Streptococcus spp*. were isolated from 373 & 219 positive (CMT) cow's quarters milk samples and buffalo's quarters milk samples, respectively. The isolates were identified biochemically as 34 (43.0%) *S. uberis*, 25 (31.6%) *S. dysgalactia*e and 20 (25.3%) other streptococci in cows' milk samples and 16 (48.5%) *S. uberis*, 10 (30.3%) *S. dysgalactiae* and 7 (21.2%) other streptococci in buffalos' milk samples. Meanwhile, PCR method revealed a total of 68 (18.2%) & 27 (12.3%) environmental Streptococci isolated from cow's quarters milk samples and buffalo quarters milk samples, respectively. 29 (42.6%) *S. uberis*, 17 (25.0%) S. *dysgalactia*e and 22 (32.4%) other streptococcus in cow's samples and 11 (40.7%) *S. ubris*, 8 (29.6%), *S. dysgalactia*e and 8 (29.6%) other streptococcus in buffalo samples. The *S.uberis* and *dysgalactiae* strains were characterized genotypically by the presence of virulence genes: plasminogen (*mig*) and streptokinase (*sk*) in *S. uberis* strains and haemolysin

(*hl*) and surface protein (*sp*) in *S. dysgalactia*e strains. *S. uberis* strains expressed these genes were (3.4 % and 6.9%) in cows' isolates and it was (12.5% and 0%) in buffalos' isolates, respectively. *S. dysgalactiae* strains which have these genes were (11.8 % and 0%) in cows' isolates meanwhile it was (18.2% and 0%) in buffalos' isolates, respectively. Virulence profiles were identified on the basis of the combination of virulence factors. Bovine *S. uberis* and *S. dysgalactia*e isolates causing SCM included in the present study showed pathogenicity in regard to their genotypic characteristics.

Key words:

Bovine subclinical mastitis, Environmental streptococci, PCR and virulence genes.

INTRODUCTION

Mastitis, inflammation of the mammary gland, can be caused by a wide range of organisms, including Gram-negative and Gram-positive bacteria, mycoplasmas and algae. Many microbial species that are common causes of bovine mastitis, such as *Escherichia coli, Klebsiella pneumoniae, Streptococcus agalactiae* and *Staphylococcus aureus* may occur as commensals or pathogens of humans, whereas other causative species, such as *Streptococcus uberis* and *Streptococcus dysgalactiae* subsp are almost exclusively found in animals. **(Ruth *et al.,* 2011)**.The term "environmental streps." often is used to replace the terms" non-agalactia streptococci" and refers to all forms of streptococcal bacteria other than *Streptococcus agalactiae* that are capable of causing mastitis in dairy cows. The most two common forms of environmental streptococci are *S.dysgalactiae* and *S.uberis.* This study will focus mainly on *S.uberis* and *S. dysgalactiae* infections as they are the environmental streptococcus bacteria that commonly found in dairy herds. *S.uberis* is one of the most important environmental pathogens implicated in bovine mastitis, accounting for a significant proportion of subclinical intramammary infections **(Phuektes *et al*., 2001**) **and (Poelarends *et al.* 2001**). Severe economic impact is caused by subclinical infections, which may hamper the control of mastitis because they often go unnoticed and untreated, resulting in long duration of the infection **(Wilson *et al.*, 1997**). Moreover, economic losses are also caused as result of the failure to eradicate *S. uberis* mastitis through management that controls transmission of contagious mastitis **(Leigh 1999)**. Several potential *S. uberis* virulence factors have been proposed, including plasminogen activator factor (PAF) **(Rosey *et al*., 1999**), streptokinase (SK), haemolysin (HL) hyaluronidase (HYA) **(Oliver *et al*., 1998**) **and**

(**Schaufuss *et al*., 1989**). Molecular studies have yielded evidence for the predominance of particular strains in some herds suggesting that it could be the result of differences between strains in pathogen virulence **(Baseggio *et al*., 1997) and (Phuektes *et al*., 2001**). The epidemiology of *S. dysgalactiae* subsp. *dysgalactiae* is poorly understood. It has been described as a contagious pathogen **(Fox, 1993**) and as an environmental pathogen **(Sommerhäuser *et al.*, 2003)**, but environmental sources have not been investigated. Evidence for the dual nature of this pathogen comes from intervention studies conducted in the 1960s **(Neave *et al*., 1969**) and from molecular studies conducted in the 1990s. The focus of this paper is part of "molecular epidemiology", which we interpret as the use of DNA-based characterization of micro-organisms at the Gram positive environmental streptococcus subspecies level to understand their virulence characteristics, and to a review of use of molecular epidemiology in veterinary practice for applications and interpretations.

MATERIAL AND METHODS

This study was carried out with 304 lactating animals (169 cows and 135 buffaloes) in Kafr El-Sheikh Governorate. 1226 quarter milk samples, 653 cow quarters and 531 buffaloes' quarters in addition to 46 (23&9 cows´ and buffalos' blind quarters)] were collected from apparently healthy udder of lactating animal with normal milk. Milk samples were cultured and bacterial isolates were identified as environmental streptococci with specific biochemical tests and PCR. Also the selected virulence genes were confirmed in the identified strains by molecular technique.

In a clean environment, thoroughly wiping the teats with 70% ethyl alcohol with paying extra attention to teat orifice was applied. After discarding the first few milk squirts, a milk samples were subjected to the CMT **(Schalm *et al.*, 1971)**.

CMT-positive quarters' milk samples were collected under aseptic conditions in labeled sterile screw caped bottles. According to the visible reaction of the CMT, the results were classified into four scores: 0 = negative or traces (no change in consistency), 1 = slightly positive (+), 2 = positive (++) and 3 = highly positive (+++). Scores 1, 2 and 3 depend on the degree of gelation that were indicated by gelatinous mass **(Schuppel and Schwope, 1998).** Ten milliliters of the each positive CMT milk samples were centrifuged. The sediment was suspended with equal volumes of sterile distilled water. Loopful from the prepared milk samples were streaked on Edward agar and incubated at 37 °C for 48 h. Colonies were

identified by morphology, cultural characters and biochemical as well as by molecular means **(Cruickshank, 1970) and (Quinn *et al.*, 2011).**

A boiling procedure was used to extract DNA from selected bacterial strain according to **(Reischl *et al.*, 1994).** Extracted DNA were examined for detection of 16S gene, (gene encoding species specific for streptococci), and some virulence genes for 2 major isolates species (*S. uberis* and *S. dysgalactiae*) as (mig and skc genes) and (*pau, and hl* genes) were performed **(Johnsen *et.al.*, 1999).**

All assays were carried out by using total volume of 25ul reaction mix contain 5ul of template DNA, 20 pmol of each primer (Metabion international AG, Germany), and 1X of PCR mix (PCR Master Mix, Fermentas, Life Science). The PCR cycles were carried out in Eppendorf AG (22331 Hamburg) thermocycler. Detailed sequences of primers and cycling protocols are depicted in (Table 1), In (Table 1), the analysis of PCR products was carried out using 1.5 % ethedium bromide stained agarose.

Table (1):primer sequences, target genes and cycling profiles of PCR assays used in the study.

S	Primers	PCR program		Sequence (5′–3′)	Size (bp)	Reference
1	Streptococcus spp.	94ºC/2m 55ºC/2m 72ºC/1m	30 cycles	16s rRNA F: GAT ACA TAG CCG ACC TGA GA 16s rRNA R: AGG GCC TAA CAC CTA GCA CT	561	Pradhan *et al.* (2011)
2	*dys*	94ºC/2m 55ºC/2m 72ºC/1m	30 cycles	16S-23S rDNA ISR F: TGGAACACGTTAGGGTCG 16S-23S rDNA ISR R: CTTTTACTAGTATATCTTAACTA	270	Forsman *et al.* (1997).
3	*sub*	94 °C/30 s 55 °C/30 s 72 °C/30 s	35 cycles	16S-23S rDNA ISR F: TAAGGAACACGTTGGTTAAG 16S-23S rDNA ISR R: TCCAGTCCTTAGACCTTCT	330	Forsman *et al.* (1997).
4	*sk*	94 °C/30 s 52 °C/60 s 72 °C/30 s	35 cycles	5′-CTC CTC TCC AAC AAA GAG G- 3′ SKC-II 5′-GAA GGC CTT CCC CTT TGA AA- 3′	475	Johnsen *et. al.* (1999)
5	*pau*	94ºC/45s 54ºC/60s 72ºC/60s	30 cycles	5′-AAT AAC CGG TTA TGA TTC CGA CTA C- 3′ 5′-AAA ATT TAC TCG AGA CTT CCT TTA AGG- 3′	439	Rosey *et al.* (1999)
6	*hl*	94ºC/45s 57ºC/60s 72ºC/80s	35 cycles	Forward: GCC AAA GCC GAA TCT AAG Reverse: CGC ATA TAC ATC CCA TGG C	248	Shunkhnand *et al.* (2005)
7	*mig*	94ºC/2m 55ºC/2m 72ºC/1m	30 cycles	Forward: CGTTTTTAGTTTCGGGAGCA Reverse: TGCCTTCAATTGAGTCTGCTG	950	Akineden *et al.* (2001)

All PCR programs started with initial denaturation at 94°C for 4 minutes and were ended with final extension at 72°C for 10 minutes.

RESULTS

Table (2): Relation between positive CMT scores and degree of quarter attack.

Animal Spp.	No. of Animals	Blind Quarters	Examined Quarters	Total positive CMT		CMT categories					
						CMT 1		CMT 2		CMT 3	
				No.	%	No.	%	No.	%	No.	%
Cows	169	23	653	373	57.1	75	20.1	122	32.7	176	47.2
Buffaloes	135	9	531	219	41.2	94	42.9	81	37.0	44	20.1

California mastitis test (CMT).

Table (3): Incidence of environmental streptococci isolated from positive CMT of cows' milk samples based on biochemical and PCR tests.

Test type	Total positive environmental streptococci n (373)		Types of isolated environmental streptococci based on biochemical tests					
			S. uberis		*S. dysgalactiae*		Other environmental streptococci	
	No.	%	No.	%	No.	%	No.	%
Culture	79	21.2	34	43.0	25	31.6	20	25.3
PCR	68	18.2	29	42.6	17	25.0	22	32.4

Table (4): Incidence of environmental streptococci isolated from positive CMT of buffaloes' milk samples based on biochemical and PCR tests.

Test type	Total positive environmental streptococci n (219)		Types of isolated environmental streptococci based on biochemical tests					
			S. uberis		*S. dysgalactiae*		Other environmental streptococci	
	No.	%	No.	%	No.	%	No.	%
Culture	33	15.1	16	48.5	10	30.3	7	21.2
PCR	27	12.3	11	40.7	8	29.6	8	29.6

Table (5): occurrence of virulence genes in some environmental streptococcal isolates in cows and buffaloes milk samples.

Target of Streptococcus *genes*	Environmental Streptococcus strains	Oligonucleotide Primer	Cow samples		Buffaloes Samples	
			No.	%	No.	%
Heamolysin	*S. dysgalactiae*	(*hl*) gene	2/17	11.8	2/11	18.2
Surface expressed protein		*(mig) gene*	0	0	0	0
Plasminogen	*S. uberis*	(*pau*) gene	1/29	3.4	1/8	12.5
Streptokinase		(*sk*) gene	2/29	6.9	0	0

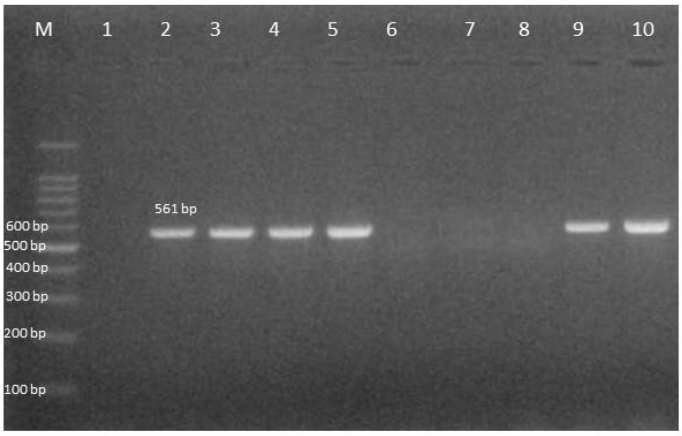

Fig. (1): Electrophoresis gel show the results of PCR amplification of *Streptococcus spp.* gene (561 bp) analyzed on 1.5% agarose gel, Lane M: 100 bp DNA ladder; PCR amplified 561 bp product of *Streptococcus spp.* Positive Lanes 2,3,4, 5,9, and 10. Lanes 1, 6, 7, and 8 were negative.

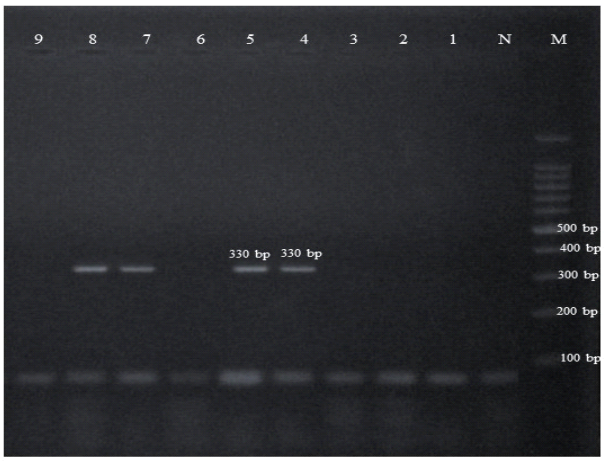

Fig. (2): Electrophoresis gel show the results of PCR amplification of *(sub)* gene of *S. uberis* gene analyzed on 1.5% agarose gel, Lane M: 100 bp DNA ladder; Lane N: negative control; PCR amplified 330 bp product of *S. uberis* Positive Lanes 4, 5, 7, and 8. Lanes 1, 2, 3, 6 and 9 were negative.

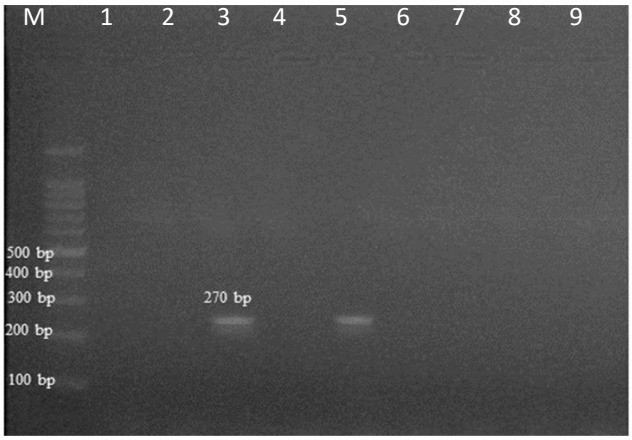

Fig. (3): Electrophoresis gel show the results of PCR amplification of (*dy*s) gene of *S. dysgalactia*e gene analyzed on 1.5% agarose gel, Lane M: 100 bp DNA ladder; PCR amplified 270 bp product of *S. dysgalactiae* Positive Lanes 3 and 5. Lanes 1, 2, 4, 6, 7, 8 and 9 were negative.

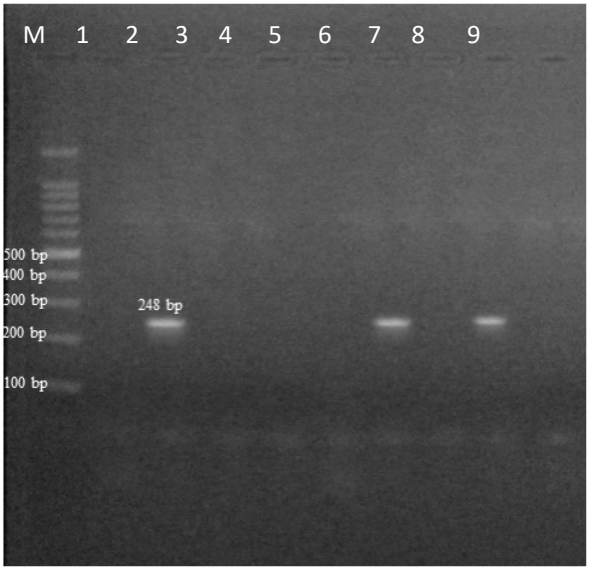

Fig. (4): Electrophoresis gel show the results of PCR amplification of haemolysin gene *(hl)* of *S. dysgalactiae* gene analyzed on 1.5% agarose gel, Lane M: 100 bp DNA ladder; PCR amplified 248 bp product of *S. dysgalactiae* Positive Lanes 2, 7 and 9. Lanes 1, 3, 4, 5, 6 and 8 were negative

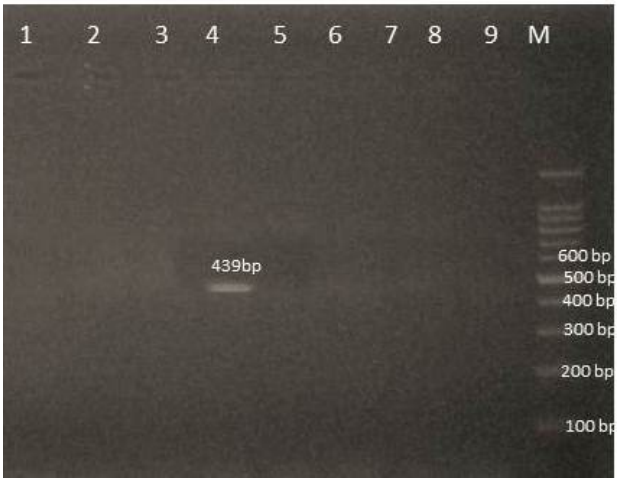

Fig. (5): Agarose gel electrophoresis of products on a 1.5% agarose gel from PCR of plasminogen activator (*pau)* gene, Lane M: 100 bp DNA ladder; Lane 4: PCR amplified 439 bp product of (*pau*) gene Positive; others lanes were negative.

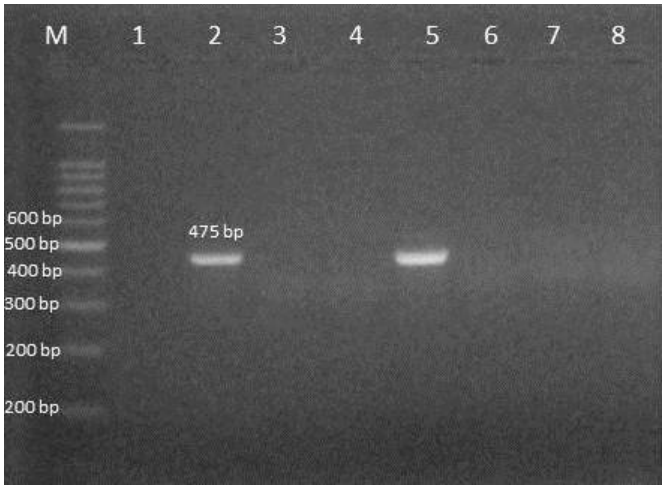

Fig. (6): Agarose gel electrophoresis of products on a 1.5% agarose gel from PCR of Streptokinase gene *(sk)*, Lane M: 100 bp DNA ladder; Lane: PCR amplified 475 bp product of *sk*c gene Positive; Lanes 2-5 and other lanes are negative.

DISCUSSION

Mastitis, particularly the subclinical type, is one of the most persistent and widely spread disease conditions of importance to milk hygiene and quality among dairy cattle worldwide **(Coulon *et al*., 2002)**. *S. uberis* and *S. dysgalactiae* are well-recognized worldwide environmental agents of subclinical mastitis in bovines. In Kafr El-Sheikh Governorate, Egypt, data on virulence factors profiles and PFGE patterns of these microorganisms are not available. In the present study, we investigated the genotypic characteristics of *S. uberis and S. dysgalactiae* isolates from cows and buffaloes with subclinical mastitis in dairy farms of the Kafr El-Sheikh Governorate, a highly populated area where many of the most important dairy farms are located. In this investigation, studies were run on subclinical mastitis for totally 169 dairy cows as well as 135 dairy buffaloes distributed in different farms, along a whole season through field screening surveys by using of the CMT for each quarter milk sample. As showed in (Table 2) of 653 quarters of cows' milk, 373(57.1%) were positive CMT, classified into three categories as follow: 75 (20.1%) one plus (CMT 1), 122 (32.7%) two plus (CMT 2) and 176 (47.2%) three plus (CMT 3). These results were higher than results reported by **(Ahmed and Mohammed, 2010)** which could be attributed to infected farms zone as well as bad management and poor hygiene. In case of buffalo milk of 531 quarters examined, 219 (41.2%) were CMT positive. These results were less than that of cows, quarters and this is may be due to high defense mechanisms of buffalo udders. This result was divided into 94

(42.9%), 81 (37%) and 44 (20.1%), represented CMT1, CMT2 and CMT3, respectively. The high prevalence of CMT positive cases were agreed with **(Ismail and Hatem, 1998**) in Egypt under nearly same condition of management. Also **(El-Balkemy *et al.*, 1997)** reported nearly the same results. Many investigations **(Dingwell *et al.,* 2003 and Milne *et al.,* 2003)** had assured that bacteriological culture is the gold standard method for identifying intra mammary infection (IMI) but till nowadays the bacteriological sampling is not feasible as a routine test to identify subclinical mastitis so we need to do molecular identification as a more accurate and sensitive test specially for identification of environmental streptococci. In (Table 3) total culture test results of environmental streptococci in cow's milk showed higher percentage (21.2%) than PCR results (18.2%), this is a logic result due to the accuracy, sensitivity and specificity of molecular identification. Also *S.uberis* reported an elevated rate (43%) than *S. dysgalactiae* (31.6%) from culture results, meanwhile it was (42.6%) and (25%) from PCR results in *S.uberis* and *S. dysgalactiae,* respectively. On the other hand, our investigation detected that other environmental streptococci were isolated in the % of (25.3%) and (32.4%) by culture and PCR investigations respectively. Nearly the same results were detected by **El-Attar *et al.* (2002) and Dego and Tareke (2003)**. In (Table 3) the present result was (15.1%) and (12.3%) as a total positive environmental streptococcus in culture and PCR, respectively. These results were divided to (48.5%) and (30.3%) for *S.ubris* and *S. dysgalactia* in culture results, meanwhile it was (40.7%) and (29.6%) for both strains in PCR results, respectively. On the other hand, our investigation gave result of (21.2%) and (29.6%) for other environmental streptococci reported by culture and PCR techniques. All these results were in accordance with many studies applied also in Africa. In southern Ethiopia, **(Dego and Tareke, 2003)**. In Tanzania **(Mdegela *et al.,* 2005**) found traditional animals more resistant for environmental streptococcal infection than imported dairy animals. In Algeria, **(Ghazi and Niar, 2006**) found that local buffalo breed is more resistant compared to the imported cow's ones for the intra mammary infection. However, more studies are needed to shed more light on this differential udder infection rates between local and imported breeds. In (Table 4) our results obtained from buffalo milk samples were (15.1%) and (12.3%) as a total positive environmental streptococcus in culture and PCR respectively. These results were divided to (48.5%) & (30.3%) for *S. uberis* and *S. dysgalactiae* in culture results, meanwhile they were (40.7%) & (29.6%) for both strains in PCR results, respectively.

On the other hand, this investigation gave isolation rate of (21.2%) & (29.6%) for other environmental streptococci reported by culture and PCR techniques, respectively. All these results were in accordance with many studies applied also in Africa. In southern Ethiopia, **(Dego and Tareke (2003)**. In Tanzania **(Mdegela *et al.* 2005)** found traditional animals more resistant than imported dairy animals. In Algeria, **(Ghazi and Niar 2006)** found that local buffalo breeds were more resistant compared to the imported cow's ones**,** however, more studies are needed to shed more light on this differential udder infection rates between local and imported breeds. (Table 5) collected the results of occurrence of virulence genes in *S. uberis* and *S. dysg*alactiae isolates in cows and buffaloes milk samples, in which incidence of *sk* gene *pau* gene were 2/29 (6.9%), 1/29 (3.4%) examined, respectively, these results of plasminogen activator factor **(Rosey *et al.,* 1999)**. While incidence of *sk* gene were not detected from the twenty-nine samples, but (*pau*) gene incidence was1/8 (12.5%) in buffalo's samples. Incidence of *S. dysg* virulence gene *(mig)* were not detected from the seventeen samples, while *hl* gene incidence was 2/17 (11.8%) in cow's samples, while incidence *mig* gene not detected from the eleven samples (*hl*) gene 2/11 (18.2%) in buffalo's samples, these results of streptokinase (*SK*), hemolysis (*hl*) in agreement with **Oliver *et al.* (1998) and Schaufuss *et al.* (1989).**

CONCLUSION

Controlling *S. uberis* and *S. dysgalactia* infections remains an important task causing subclinical mastitis.This study makes stress and put high light on these microorganisms as un-intentioned and a hidden cause of udder problems.

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REFERENCES

Ahmed Abdel-Rady and Mohammed Sayed (2010): Epidemiological studies on subclinical mastitis in dairy cows in Assiut Governorate Veterinary World, vol.2 (10):373-380.

Baseggio, N.; Mansell p.; Browning, J. and Browning, G. (1997): Strain differentiation of isolates of streptococci from bovine mastitis by pulse-field gel electrophoresis. Mol and Cell Probes;11: 349-54.

Calvinho, I.F.; Tirante, L.I. and Prevalencia, D.E. (2005): Microorganismos patógenos de mastitis bovina y evolución Del estado de salud de la glándula mamaria end argentina end los últimos 25 años. Revista Fave- Ciencias Veterinarias; 4: 1-12.

Costa, E.O.; Ribeiro A.R.; Watanabe E.T. and Melville, P.A. (1998): Infectious bovine mastitis caused by environmental organisms. Zentralbl veterinarmed [b] 45: 65-71.

Coulon, J.B.; Gasqui, P.; Barnouin, J.; Ollier, A.; Pradel, P. And Pomiès, D. (2002): effect of mastitis and related-germ on milk yield and composition during naturally-occurring udder infections in dairy cows. Anim. Res. 51: 383-393.

Cruickshank, R. (1970): Medical Microbiology. 11th Ed. The English Language Book Society E. and Livingston Ltd.

Dego, O.K. and Tareke, F. (2003): bovine mastitis in selected areas of southern ethiopia. Trop. Anim. Health prods. 35:197.

Dingwell, R.T.; Leslie, K.E.; schukken, Y.H.; Sargeant, J.M. and Timms, L.L. (2003): evaluation of the California mastitis test to detect an intramammary Infection with a major pathogen in early lactation dairy cows. *Can. Vet. J.* 44:413.

V.L Douglas, Fenwick, S.G; Pfeiffer, D.U.; Williamson, N.B. and Holmes, C.W. (2000): Genomic typing of *streptococcus uberis* isolates from cases of mastitis, in new zealand dairy cows, using pulsed-field gel electrophoresis. Vet microbial. 75: 27- 41.

El-attar, A.A.; Salama, M.E. and Abd El-Samie, M.M. (2002): Incidence of mastitis in lactating cows and buffaloes kept under different managemental conditions in ismailia province. Vet. Med. J. 2:583.

El-Balkemy, F.A.; Esmat, M.; Menazie, Afaf; Farag, Azza N. (1997): evaluation of screening tests used for detection of subclinical mastitis. 4th sci. Cong. Egyptian Society for cattle diseases, Assiut, Egypt. Pp 181.

Fox, L.K. And Gay, J.M. (1993): Contagious mastitis. Vet Clin North Am Food Anim Pract. ; 9 (3):475-87.

Ghazi, k. and Niar, A. (2006): Incidence of mastitis in various bovine breedings in tiaret area (Algeria). Assiut Vet. Med. J. 52:198.

Gianneechini, R.; Concha, C.; Rivero, R.; Delucci, I.; Moreno and L.and López, J. (2002): Occurrence of clinical and sub-clinical mastitis in dairy herds in the west littoral region in Uruguay. Acta Vet Scand; 43: 221-30.

Ismail, T.M. and Hatem, M.E. (1998): prevalence of subclinical mastitis in a dairy cattle herd in the eastern region of Saudi Arabia. The 8th sci. Con. Fac. Vet. Med. Assiut uni. Egypt.

Jayarao, B.M.; Gillespie, B.E.; Lewis, M.J.; Dowlen, H.H. and Oliver, S.P. (1999): Epidemiology of *streptococcus uberis* intramammary infections in a dairy herd. J. Vet Med; 46: 433 - 42.

Johnsen, L. B., K. Poulsen, M.; Kilian, and T. E. Peterson. (1999): Purification and cloning of a streptokinase from *Streptococcus uberis*. Infect. Immun. 67:1072-1078.

Leigh, J.A. (1999): *Streptococcus uberis*: a permanent barrier to the control of bovine mastitis, Vet J. 157: 225-38.

Mdegela, R.H. (2005): Mastitis in smallholder dairy and pastoral cattle herds in the urban and periurban areas of the Dodoma municipality in central Tanzania. Livestock res. Rural develops. 17:11.

Milne, M.H.; Biggs, A.M.; Fitzpatrick, J.L.; Innocent, G.T. and Barrett, D.C. (2003): Use of clinical information to predict the characteristics of bacteria isolated from clinical cases of bovine mastitis. Vet. Rec. 152:615.

Mirta, C.; Lasagno,L.; Elina, B.; Reinoso,L.; silvana ,A.; Dieser,L.; Iluis,F. Calvinho,M.; Fernanda, A; Buzzola,B; Claudina,L; Vissio,F; Cristina ,J.; Bogni,L.; Liliana, M.and Odierno L.L. (2011): phenotypic and genotypic characterization of *streptococcus uberis* isolated from bovine subclinical mastitis in argentinean dairy farms, revista argentina de microbiología 43: 212-217.

Neave, F.K., Dodd, F.H., Kingwill, R.G. and Westgarth, D.R. (1969): Control of mastitis in the dairy herd by hygiene and management. J. Dairy Sci.; 52:696-707.

Oliver, S.; Almeida, R. and Calvinho L. (1998): Virulence factors of *streptococcus uberis* isolated from cows with mastitis. J vet med b; 45: 461-71.

Oliver, S.P.A; B.E.; Gillespie, A. and Jayarao, B.M. (1998): Detection of New and Persistent *Streptococcus uberis* And *Streptococcus dysgalactiae* Intramammary Infections By Polymerase Chain Reaction-Based DNA ¢Ngerprinting, FEMS Microbiology Letters 160 -69^73.

Payne, W.J.A. and Wilson, R.T. (1999): an introduction to animal husbandry in the tropics. 5th ed. Blackwell Publishing Ltd, Iowa State University press USA pp 826.

Phuektes, P.; Mansell, P.D.; Dyson, RS; Hooper, N.D; Dick, J.S. And Browning, G.F. (2001): Molecular epidemiology of *Streptococcus uberis* isolates from dairy cows with Mastitis. J Clin Microbial. 39: 1460-6.

Poelarends, J.; Hogeveen, H.; Sampimon, O. and Sol, J. (2001): Monitoring subclinical mastitis in dutch dairy herds. In: Proc Natl Mast. Counc, Vancouver. P 145-9.

Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carter, G.R. (2011): Clinical Veterinary Microbiology. 1st Edn, London, Mosby. PP: 22-91.

Reischl, U. Pluz, M. Ehret, W. and Wolf, H. (1994): PCR-based detection of ycobacteria in sputum samples using a simple and reliable DNA extraction protocol. In Bio. Techniques, 17: 844 -845.

Rosey, E.; Lincoln, R.; Ward, R.; Vancey, R.; Leigh, J.and Pau, A. (1999): A novel plasminogen activator from *streptococcus uberis*. Fems microbiol let. 178: 27-33.

Ruth, N.; Zadoks; John, R.; Middleton; Scott Mcdougall, Jorgen katholm; Ynte, H.and Schukken (2011): molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. J mammary gland Biol Neoplasia. 16:357-372.

Schalm, O. W.; Carrol, C. and Jain, N. C. (1971): Bovine mastitis. 1st Ed. Lea and Febiger, Philadelphia, USA.

Schaufuss, P.; Sting, R.; Schaeg, W. and Blobel, H. (1989): Isolation and characterization of hyaluronidase from *streptococcus uberis*. Zentralbl Bakteriol. 271: 46-53.

Schuppel, H. And Schwope, M. (1998): diagnosis of mastitis using california mastitis test and measurement of electric conductivity. Archiv Für Lebensmittel Hygiene, 49:61.

Sommerhäuser, J.; Kloppert, B.; Wolter, W.; Zschöck, M.; Sobiraj, A. and Failing, K. (2003): The epidemiology of *Staphylococcus aureus* infections from subclinical mastitis in dairy cows during a control programme. Vet. Microbiol. 96:91-102.

Struelens, M. J. A.; Bauernfeind, A.; Van Belkum, D.; Blanc, B.D.; Cookson, L.; Dijkshoorn, N.; El Solh, J.; Etienne, J.; Garaizar, P.; Gerner-Smidt, N.; Legakis, H.; De Lencastre, M.H.; Nicolas, T.L.; Pitt. Römling, U.; Rosdahl, V. and Witte.W. (1996): Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems. Clin Microbiol infect.2:2-11.

Tenover, F. C., R. D.; Arbeit, R. V.; Goering, P. A.; Mickelsen, B. E.; Murray, D. H.; Persing, and Swaminathan,B. (1995): Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J.Clin.Microbiol. 33: 2233-2239.

Tassios,>[V.; [Haeggman,[L.; Cookson,[S.;[Fry,B.;[Fussing,[NK.; Green,V.; Feil,J.; Gerner-Smidt, E.; Brisse, P.; and Struelens,S.[M.(2007): European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group on Epidemiological Markers (ESGEM): Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin Microbiol Infect. Oct; 13 Suppl 3:1- 46.

Wilson, DJ; Gonzalez, M. and Das H.H. (1997): Bovine mastitis pathogens in new york and Pennsylvania: prevalence and effects on somatic cell count and milk production. J Dairy Sci 80: 2592-8.