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BACTERIOLOGICAL AND MOLECULAR INVESTIGATIONS ON CAPRINE MASTITIC MILK WITH SPECIAL REFERENCE ON *Corynebacterium pseudotuberculosis*

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ABSTRACT

The aim of the present study aimed to estimate the prevalence of caprine mastitis with emphasis on *Corynebacterium pseudotuberculosis* mastitis in Egyptian dairy goats in s farms. Three hundred thirty-six half milk samples were collected from one hundred seventy seven dairy goats of various crossbreeds, in mid to late lactation period after clinical examination**.** Somatic Cell Count (SCC) and California Mastitis Test (CMT) were assessed in 246 normal half milk samples.One-hundred eighty milk samples (90 clinical and 96 subclinical) were subjected to bacteriological examination, the identified isolets *C. pseudotuberculosis* were further confirmed by molecular diagnosis of *pld* and *rpo*B genes by PCR. Prevalence of clinical mastitis was 30.5 % (54 animals), while 69.5 % (123 animals) were clinically healthy with normal milk secretion. Out of the selected 246 clinically healthy half milk samples, 96 milk samples (39%) showed subclinical mastitis as detected by SCC (SCC ≥1,000,000 cells/ml) and California mastitis test scored (+++). The most prevalent bacteria detected in this study were *Coagulase negative staphylococci (CNS)* 87/186 (46.8 %)*, Staphylococcus aureus* 53/186 (33.9%), *E. coli* 31/186 (16.7 %), *Streptococcus spp*. 23/186 (12.4 %) and *C. Pseudotuberculosis, 24/186* (12.9%). Molecular detection of *C. Pseudotuberculosis* virulence genes revealed that gene coding for *pld* was evidenced in 16 samples of 24 bacteriologically diagnosed samples as *C. pseudotuberculosis* (66.7%), while gene coding for *rpo*B was detected in 6/24 samples (25%).

Keywords:

Bacteriological investigation, molecular investigation, caprine mastitis, *Corynebacterium pseudotuberculosis.*

INTRODUCTION

The last two decades have seen intensification in dairy goat production with significant increase in the number of goats worldwide **(Skapetas and Bampidis 2016).** The nutritional qualities of goat milk are similar to human milk and it is less allergenic for human than bovine milk **(Haenlein 2004 and Park and Haenlein 2008)**. Mastitis is the most serious and costly disease in dairy goats. This is due to financial losses attributed to its negative impact on milk quantity and components **(Silanikove *et al*., 2010; Barron-Bravo *et al*. 2013 and Jimenez-Granado *et al*., 2014)**, . Also it is the most frequent cause of culling for sanitary reasons **(Leitner *et al*., 2008a and Marogna *et al*., 2010)**.In dairy goats, incidence of clinical mastitis may not exceed 5 %, while subclinical mastitis (SCM) is common and about 6 times the incidence of clinical affections **(Moroni *et al*., 2005; Contreras *et al*., 2007 and Leitner *et al*., 2007).** Mastitis in dairy goats is usually **associated** with production loss, downgrading of milk quality and hygiene, increased replacement cost, and considerable veterinary expenses **(Koop *et al*., 2010).** Dilution effect of the 4 quarters in cows diminishes projection effect of SCM on bulk milk in the infected glands **(Pitkala *et al*., 2004 and Leitner *et al*., 2008b).** However, it is relatively high in sheep and goats compared with cows because of the strong immune response to the infection and the existence of only two mammary glands **(Leitner *et al*., 2011).** In dairy goats, the problem of subclinical mastitis is exacerbated by the fact that infected goats demonstrate neither udder symptoms nor abnormal milk, hence identification of disease is delayed **(Haenlein 2002).** Thus, subclinical mastitis in goats should be considered as a serious economic burden both by farmers and by the dairy industry **(Silanikove *et al*., 2014).** Consequently, other diagnostic methods, such as indirect measurements of somatic cell count with the California Mastitis Test (CMT) were developed **(Raynal-Ljutovac *et al*. 2007; Viguier *et al*. 2009 and Persson and Olofsson 2011).** SCC has been commonly used worldwide as an indicator for subclinical mastitis, also to assess the efficiency of mastitis control programs in dairy cattle and buffalo **(Schukken *et al*., 2003).** Unfortunately, it is difficult to interpret in goats, as in goats the relationship between bacterial infections and a SCC value is not as simple as in dairy cattle, since non-infectious factors have a big impact on SCC. As well as, other intrinsic factors like time and number of lactation and prolificity, per day affect SCC. In addition, milking routine, seasonality and

food affect SCC **(Paape and Capuco 1997 and Schaeren and Maurer 2006).** In addition, milk secretion in goats is mostly apocrine and therefore characterized by the presence of epithelial debris or cytoplasmic particles, which makes the use of DNA specific counters mandatory **(Jimenez-Granado *et al.*, 2014).** Mainly SCM in goats is caused by *Staphylococcus aureus* (*S. aureus*), coagulase-negative staphylococci (CNS), *Streptococcus agalactiae*, Streptococcus Group C. and *Mycoplasma spp*. **(Bagnicka *et al*., 2011 and Persson and Olofsson, 2011).** *C. pseudotuberculosis* is one of the infectious causative agents of mastitis occasionally encountered in sheep and goats, and is most likely to represent an extension of infection from the adjacent supra-mammary lymph node **(Bagnicka *et al*., 2011 and Hristov *et al*., 2016).** *C. pseudotuberculosis* is the etiologic agent of caseous lymphadenitis (CLA) **Brown and Olander (1987),** characterized by the formation of chronic abscesses in several organs in small ruminants **(Williamson; 2001)**. The disease is worldwide distributed **Paton *et al*; (2003) and Guimares *et al*., (2011)** and causes considerable financial losses in the goat and sheep industry due to decreased milk production, wasting, low reproductive rates, and condemnation of carcasses because of internal abscesses **(Arsenault, *et. al.*, 2003 and Dorella *et al*., 2006).** Although rare, *C. pseudotuberculosis* has public health significance, causing human lymphadenitis, frequently similar to those observed in sheep and goats (CLA), and acquired after close contact with an infected animal **(Join-Lambert *et al.* 2006 and Hemond *et al*. 2009).** Recently, molecular diagnosis of pathogens has been introduced. PCR has been explored as rapid and sensitive approach for diagnosis of mastitis-causing pathogens **(Koop *et al.* 2012).** The most important virulence determinant identified in *C. pseudotuberculosis* is phospholipase D (Pld) **(Hodgson *et al.*, 1999).** *PLD* gene encodes the phospholipase D - *PLD* exotoxin, an enzyme that catalyzes the dissociation of sphingomyelin and increases vascular permeability. This leads to the spread and survival of *C. pseudotuberculosis* in the cells, and consequently the invasion of the body and transport by phagocytes to regional lymph nodes **(Hodgson *et al.*, 1994 and Baird and Fontaine, 2007).** More recently, analysis of partial gene sequences from the β-subunit of RNA polymerase (*rpo*B) has been used for the identification of *Corynebacterium* spp. than analyses based on 16S rDNA. Such method has also been successfully used to identify mycobacterial species **(Kim *et al*., 1999)**. Although the *rpo*B gene is a powerful identification tool, many authors propose that it may be used to complement the 16S rRNA gene analysis in

the phylogenetic studies of Corynebacterium and Mycobacterium species **(Dorella *et al*., 2006 and Pacheco *et al*., 2007)**. However, it is mostly expensive, time-consuming. In addition, milk culture may yield no bacteria from truly infected glands with very low numbers of pathogens or due to inhibitory effect of residual antimicrobials **(Cai *et al*. 2003).** Therefore the aim of the present study that was carried on some farms is to estimate the prevalence of udder infections with emphasis on *C. pseudotuberculosis* mastitis in Egyptian dairy goats and by traditional and molocular method.

MATERIAL AND METHODS

Ethical approval:

All samples were collected as per standard sample collection procedure without giving any stress or harm to the animals. The present work was approved by the ethical committee for medical research at Animal care guidelines of the General Organization for Veterinary Services.

Animals:

One-hundred seventy-seven dairy goats of various crossbreeds and located in different Governorates of Egypt, were employed in this study. All goats were in mid to late lactation at sampling and some of these animals suffered from caseous lymphadenitis with history of chronicity of infection in these farms Fig. (1). Animals were subjected to clinical examination for detection of any clinical abnormalities with special attention to the udder by visual inspection and palpation for detection of clinical mastitis according to Kelly **(Kelly 1984).**

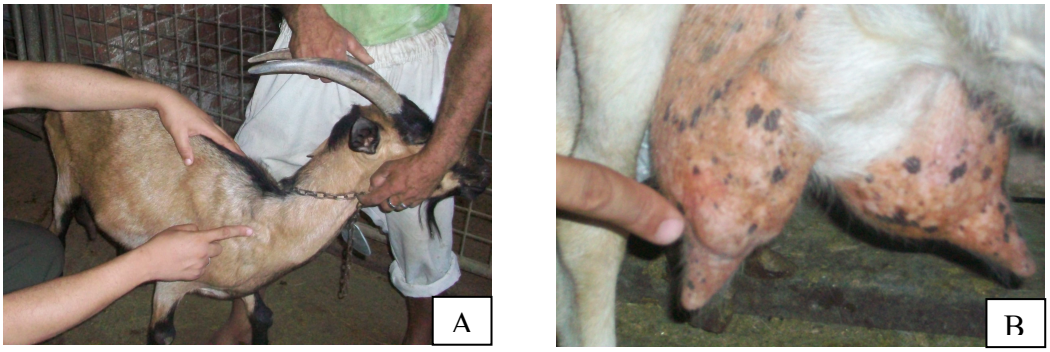

Fig. (1): Clinical examination of goats. **A:** case of gaseous lymphadenitis infection in prescapular lymph node. **B:** case of abscess in mammary gland with internal palpable abscess.

Samples:

Three hundred thirty six milk samples collected from 177 dairy goats (mastectomy was recorded in 6 halves and complete atrophy in one half was recorded in 12 animals) were employed in this study. Samples were collected from 90 halves of 54 clinically mastitic does after clinical examination and 96 samples of does suffered from subclinical mastitis collected after CMT applied for 246 apparantly normal milk samples from 123 apparantly healthy dairy goates . Milk samples were kept on icebox then transferred immediately to the laboratory of Animal Reproduction Research Institute (ARRI).

California mastits Test:

Milk samples were collected from halves of 123 does (246 half samples) with apparantly normal milk samples, just before morning milking. Using the method described by **Schalm *et al.* (1971)**. Milk halves with a CMT score of zero or + were considered healthy, whereas halves with a CMT score of ++ or +++ were considered unhealthy.

Somatic cell count:

Milk somatic cell count was assessed in 246 apparently normal half milk samples with +++ score were retested and confirmed by the NucleoCounter® SCC instrument, that is based on ChemoMetec has proven technology of Fluorescence image cytometry. This method uses the single-use SCC-Cassette™ sampling and measuring device, **(Chen *et al;* 2010).**

Bacteriological examination:

Bacteriological examination of milk samples was performed according to **Malinowski and Kłossowska (2002).** Briefly, 10 µl of milk were cultivated on blood Agar (BioMlrieux Poland), MacConkey Agar (BTL, Poland), mannitol salt agar (Oxoid Ltd, England) and Edward's medium (Oxoid Ltd, England). Plates were incubated at 37 °C and read 24 and 48 hours later. Colonies were identified by their morphology, Gram staining and biochemically. For *C. pseudotuberculosis* diagnosis, milk samples were inoculated onto brain Heart Infusion (BHI) agar supplemented with 5% defibrinated sheep blood, tenisdal medium and chocolate agar plates. The plates were incubated aerobically for approximately 48 hr. at 37°C. Colonies that morphologically resembled *C. Pseudotuberculosis* were Gram stained. Gram-positive colonies were further tested for urease activity, synergistic hemolytic activity with CAMP factor from *Rhodococcus equi* and carbohydrate fermentation (glucose, lactose, sucrose). Strains that were positive for urease and glucose fermentation, and negative for lactose and

sucrose fermentation and positive for API corynaebacterium specific test (BioMeieux - France), were identified as *C. Pseudotuberculosis* **(Rebouças *et al*., 2011).**

Molecular Diagnosis of *Corynebacterium pseudotuberculosis* (according to Pacheco *et al.*, 2007):

Extraction of DNA:

According to the above-mentioned bacteriological isolation and identification, *C. pseudotuberculosis* colonies were grown in BHI broth (BHI; Oxoid) at 37 ºC for 48 -72 hours before DNA extraction. Bacterial DNA was extracted using QIAamp DNA Mini Kit (Catalogue no.51304) according to the prescribed instructions.

Primers, amplification conditions and agarose gel electrophoresis:

The oligonucleotide primers used in this study are listed in (Table 1). Primers targeting the *pld* and *rpo*B genes of *C. pseudotuberculosis* were obtained from previously published work **(Ilhan et *al*., 2013 and Sammra *et al*., 2014).**

Amplification-reaction mixtures were prepared in volumes of 50 µL containing 5 µL of 10X PCR master mix (Fermentas, Vilnius, Lithuania), 5 µl of 25 mM MgCl2, 0.2 µL of 10 mM dNTP mixture (Fermentas), 2 U of Taq DNA polymerase (Fermentas), 1 µmol of 25 mM each primer, and 5 µL of template. PCR was performed in a DNA thermocycler (Thermo Electron Corp., Waltham, MA, USA) and amplifications were performed using protocols listed in (Table 2). The negative control contained sterile, DNase/RNase free, DEPC (Diethylpyrocarbonate)-treated water (AppliChem) instead of DNA template. As a positive control, DNA isolated from *C. pseudotuberculosis* Pl 18 strain (isolated strain from a sheep with CLA). The amplified products were analyzed by electrophoresis on a 2% (w/v) agarose gel against gel pilot 100 bp ladder (Qiagen, USA, cat. no. 239035). Amplified products were visualized using a gel documentation system and the data was analyzed through computer software. PCR products with a molecular size of 203bp *(PLD)* and 406 bp (*rpo*B) were considered positive for *C. pseudotuberculosis.*

Table (1): List of oligonucleotide primers used and their references.

Gene	Primers	Sequence (5′→3′)	PCR product	Reference
Pld	PLD-F	ATAAGCGTAAGCAGGGAGCA	203 bp	Ilhan *et al.*, 2013
	PLD-R2	ATCAGCGGTGATTGTCTTCCAGG		
*rpo*B	C2700F	CGWATGAACATYGGBCAGGT	406 bp	Sammra *et al.*, 2014
	C3130R	TCCATYTCRCCRAARCGCTG		

Table (2): Cycling conditions of the different primers during PCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
PLD	94˚C 5 min.	94˚C 30 sec.	56˚C 30 sec	72˚C 30 sec	35	72˚C 10 min.
*rpo*B	94˚C 5 min.	94˚C 30 sec.	52˚C 45 sec	72˚C 45 sec	35	72˚C 10 min.

RESULTS

Clinical examination of the udders of 177 dairy goats revealed presence of symptoms suggestive for clinical mastitis in 54 animals (30.5 %) and 123 animals (69.5%) were apparently healthy with normal milk secretion (Table 3). Clinical mastitis was considered in case of pain on milking, swelling of udder, harndness and necrosis in udder, decrease in milk production, or changes in milk.

Table (3): Results of clinical examination of 177 dairy goats.

Health status	No of animals	%
Clinical mastitis	**54**	**30.5 %**
Apparantly normal animals	**123**	**69.5 %**
Total	**177**	**100 %**

For SCC assessment, 96 milk samples from total examined 246 samples (39 %) had SC ≥ 1,000,000 cells/ml,with CMT (+++) represent subclinical mastitis, and 150 from the same number (61.0%) milk samples had SCC ≤ 1,000,000 cells/ml with CMT 0,(+) and (++) represented normal does milk samples (Table 4).

Table (4): Results of CMT and SCC estimation in 246 apparently healthy does halves milk samples**.**

SCC	California mastitis test score	No. Of does milk samples	%
SCC ≥ 1,000,000	**(+++)**	**96**	**39.0%**
SCC ≤ 1,000,000	**0,(+) and,(++)**	**150**	**61.0%**
Total		**246**	**100%**

Bacteriological examination of 186 milk samples from does suffered from clinical and subclinical mastitis revealed single infection in 63 milk samples (33.9%), mixed infection in 88 milk samples (47.3%), and 35 milk samples (18.8%) did not show any microbial growth on the utilized media (Table 5). The most predominant bacteria in this study were *coagulase negative staphylococci* (*CNS*) totally 87/186 (46.8%), it was clearly high in subclinical cases (63.5%) than clinical one (28.9%). *Staphylococcus aureus* was 53/186 (33.9%), vice versa with *CNS*, it was higher in clinical cases than subclinical (37.8% and 19.8%) respectively. *E.coli* 31/186 (16.7%) and *Streptococcus spp.*23/186(12.4%) in total manner Nearly the prevalence of infections were the same in both clinical and subclinical for *E.coli* and *Streptococcus spp.* 17.8% and 15.6%,13.3% and11.5% for both types of mastitis respectively.

Meanwhile *C. Pseudotuberculosis* was isolated and identified from 24 (12.9%) milk samples, and it was remarkably difference between clinical cases (20.0%) and subclinical mastitis (6.3%) (Table 6).

Table (5): Results of bacteriological examination of 186 does halves milk samples.

Bacteriological status	No of samples	%
No growth	**35**	**18.8%**
Single infection	**63**	**33.9%**
Mixed infection	**88**	**47.3%**
Total	**186**	**100%**

Table (6): Prevalence of pathogens causing clinical and subclinical mastitis of infected does.

Isolates	Clinical mastitis (90 samples)		Subclinical mastitis (96 samples)		Total (186 samples)	
	No.	%	No.	%	No.	%
C. Pseudotuberculosis	**18**	**20.00**	**6**	**6.3**	**24**	**12.9**
CNS	**26**	**28.9**	**61**	**63.5**	**87**	**46.8**
S. aureus	**34**	**37.8**	**19**	**19.8**	**53**	**28.5**
E. Coli	**16**	**17.8**	**15**	**15.6**	**31**	**16.7**
Streptococcus spp.	**12**	**13.3**	**11**	**11.5**	**23**	**12.4**
Total	**106**	**117.8**	**112**	**116.7**	**218**	**117.2**

Molecular detection of *C. Pseudotuberculosis* virulence genes revealed that PCR amplified DNA fragment of 203 bp and specific for the *pld* gene of *C. pseudotuberculosis* was evidenced in 16 samples of 24 bacteriologically diagnosed samples as *C. pseudotuberculosis* (66.66%) Fig. (2). While PCR amplified DNA fragment of 406 bp and specific for the *rpo*B gene of *C. pseudotuberculosis* was evidenced in 6 samples of 24 bacteriologically diagnosed samples as *C. pseudotuberculosis* (25%) Fig. (3).

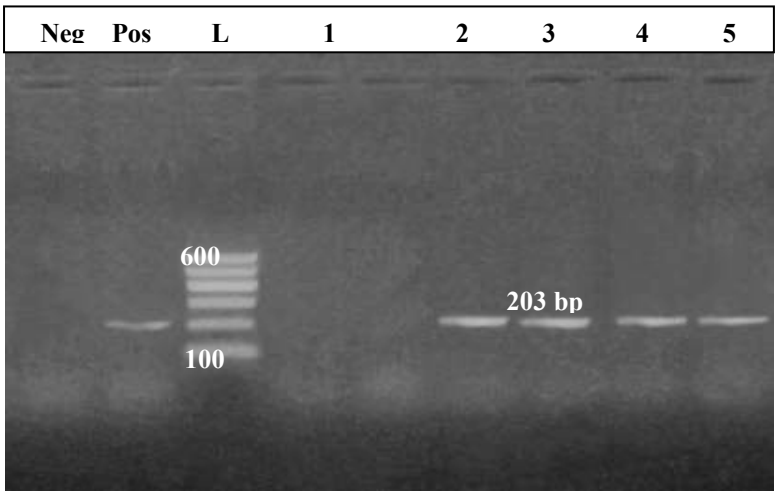

Fig. (2): PCR amplified DNA fragment of 203 bp and specific for the *pld* gene of *C. pseudotuberculosis*. Lane Neg: control negative;Lane Pos: control positive; Lane L: Lader marker; Samples 2-5 Positive samples and Sample 1 Negative sample.

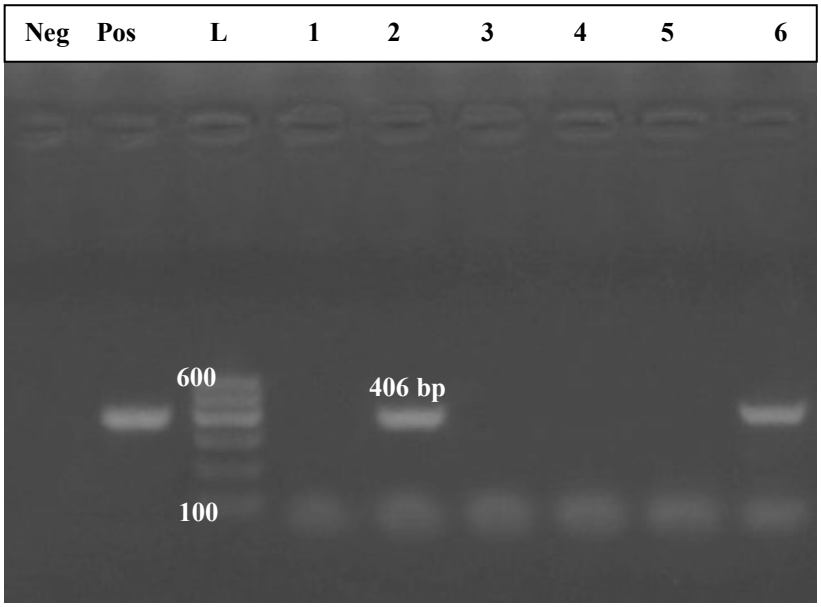

Fig. (3): PCR amplified DNA fragment of 406 bp and specific for the *rpo*B gene of *C. pseudotuberculosis*. Lane Neg: control negative; Lane Pos: control positive; Lane L: Lader marker; Lanes 2 and 6: positive Samples; Lane 1, 3, 4 and5 Negative samples.

DISCUSSION

In developing countries, mastitis is considered an important burden to the goat milk industry. As goat milk, production chain plays an important socioeconomic role. In the present study, clinical examination of the udder of 177 dairy goats revealed that 54 animals (30.5%) demonstrated clinical mastitis according to **Blood and Radostits (1989)**, and 123 animals (69.5%) were clinically healthy with normal milk secretion (Table 3).

While clinical mastitis is rather easy to detect, animals with subclinical mastitis are often difficult to find since there is a lack of reliable diagnostic methods especially at farm level. Subclinical mastitis was diagnosed by California mastitis test confirmed by somatic cell count estimation in milk secretion of 246 apparently healthy udder halves. Its incidence was 96/246 (39.0 %). The observed decreased milk yield during IMI was explained by **Petersson-Wolfe *et al*. (2013)** that an influx of neutrophils will pass between the milk-producing cells of the mammary gland and into the lumen of the alveoli resulting in damage of milk-secreting cells. The prevalence of subclinical mastitis in dairy goats has been estimated in previous studies to be 5-30% or even higher, with about 6 times the incidence of clinical mastitis **(Bergonier *et al*, 2003 and Contreras *et al*., 2003).** Others concluded that, the proportion of udder halves with subclinical IMI in goats ranged from 35 to 70 % **(Menzies and Ramanoon, 2001 and Leitner *et al.*, 2008b).** In different studies, prevalence of SCM was 36% in England **(Manser, 1986)** and 38.2% in New York **(Smith and Roguinsky, 1977).** In Brazil, the prevalence of mastitis in goats was about 75 % and most of infections were subclinical **(Peixoto *et al*., 2010).** In a recent study carried out in China, SCM was diagnosed in 45.82 % of examined dairy goats **(Zhao *et al*. 2015)**, while it was 18 % in Sweden **(Persson and Olofsson 2011)** and 30.2 % in India **(Sreeja *et al*. 2013).**

The authors attributed this high prevalence of subclinical mastitis to the poor milking hygiene and the less prevention awareness of subclinical mastitis. Poor management represented by allowing infected animals to be in contact with healthy ones, and this contaminative environment and equipment would cause a new infection. Our results concerning bacteriological findings proved single infection in 63 milk samples (33.9%), mixed infection in 88 milk samples (47.3%), and 35 (18.8%) milk samples showed no growth of any pathogenic microorganisms on our selected media (Table 5). The identified pathogens were *coagulase negative staphylococci (CNS)* 87/186 (46.8 %) and *S. aureus* 53/186 (33.9%).

C. Pseudotuberculosis was isolated and identified from 24/186 milk samples (12.9%) (Table 6). These results are to great extent in agreement with previous studies. Where, staphylococci were recorded to be the most important bacterial cause of mastitis and accounts for more than 90 % of all the isolated bacteria **(Koop *et al*. 2012 and Marogna *et al*. 2012)**. In addition, CNS was recorded to have capability of increasing somatic cell count (SCC) in goat milk, and the most prevalent class of bacteria and occurs at over 50 % in most studies of goat subclinical mastitis **(Contreras *et al.* 2007; Bagnicka *et al*. 2011 and Zhao *et al*. 2015).** Regarding Streptococci, it was reported to be the major pathogens for their sever inflammation, but they are less common in subclinical mastitis in goats **(Contreras *et al.* 2003 and Zhao *et al*. 2015).** In 2015, a similar study carried on dairy goats revealed that incidence of intramammary infection with *coagulase-negative staphylococci*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus spp.* was 59.52 %, 15.24 %, 11.43 %, and 10.95 %, respectively. The study concluded that *CNS* was the predominant pathogens **(Zhao *et al*. 2015).** Also**, Contreras *et al.*, 2007** recorded that *CNS* were the most predominant causative agent of mastitis in does. Another research group reported that *CNS* were the most predominant bacteria and encountered in 81.5% of milk samples from SCM infected does **(Salaberry *et al*., 2015).**

CNS is less pathogenic than *Staphylococcus aureus*, but produce persistent subclinical mastitis with markedly elevated somatic cell counts (SCC) **(Contreras *et al*., 1997).**

As the present study aimed to diagnose does mastitis and identify the most predominant pathogen with emphasis on *C. pseudotuberculosis*.

The organism was isolated and identified in 24 half-milk samples (12.9%), all of these samples had mixed infection mostly with CNS. Molecular diagnosis indicated that 16 isolates harbor gene sequence specific for *pld* gene Fig. (2), while gene sequence specific for the *rpoB* gene was diagnosed in six isolate only Fig. (3), while two strains did not have these two examined virulence genes. *C. pseudotuberculosis* infection results in either acute suppurative mastitis or chronic encapsulated abscesses within the mammary gland **(Valli and Parry, 1993),** causing economic losses incurred due to decreased milk production, reproductive inefficiency, condemnation of carcasses, and to a lesser extent deaths **(Burrell, 1980, Brown and Olander 1987).** *C. pseudotuberculosis* has also public health significance, causing human lymphadenitis **(Peel *et al*. 1997).**

Once infection occurs in animal, the enlarged lymph nodes and abscesses can rupture and contaminate the milk, lambs, kids, other animals and environment **(Stoops *et al*., 1984, Brown and Olander 1987).**

In previous study, prevalence of *Corynebacterium spp*. was 4.13 % in dairy goats, mostly in association with *E. coli* **(Hristov *et al*. 2016).** This is in accordance with the results reported by **Manser (1986), McDougal *et al*., (2002) and Bagnicka *et al*., (2011)**, which identify the organisms as part of the microbial agents of mastitis in goats.

The knowledge of the virulence factors involved in the mechanisms of bacterial pathogenicity in the mammary gland is important for the development of effective control and prevention of subclinical mastitis in goats. Their genes represent ideal targets for the accurate detection and identification.

To date, the most important virulence determinant identified in *C. pseudotuberculosis* is phospholipase D (*Pld*), a secreted exotoxin that possesses sphingomyelinase activity **(Hodgson *et al.,* 1990)**. *Pld* has been shown to increase vascular permeability in vivo, has dermonecrotic properties, and reduces the viability of neutrophils **(Batey, 1986 and Yozwiak and Songer. 1993)**. Studies with *C. pseudotuberculosis* strains with inactivated *Pld* have convincingly demonstrated the necessity of *Pld* for establishment of CLA **(McNamara *et al*., 1994; and Simmons *et al*., 1998)**. Mutant strains are unable to cause abscessation of the lymph nodes. Additional evidence for the importance of *Pld* in vivo comes from the observation that vaccination with formulations in which *Pld* is the major component provides protection against subsequent disease challenge **(Eggleton *et al.*, 1991).**

CONCLUSION

The present study has been directed to estimate the prevalence of udder infection in Egyptian dairy goats in the selected farms by traditional and molocular methods.

Here we describe for the probably first time the isolation and preliminary identification of *C. pseudotuberculosis* from milk of does suffering mastitis.

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