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RESEARCH ARTICLE

The Prevalence and Etiology of Subclinical Mastitis in Sheep and Goats

El-Shymaa A. Abdallah[1*], Mohamed I. Eissa[2] and Afaf M. Menaze[2]
[1]Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt
[2]Animal Medicine Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

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Abstract

From September 2016 to August 2017, four hundred and twenty four milk samples were collected from mammary halves of 212 apparently healthy lactating animals (108 ewes and 104 does) from small private flocks in different localities at Sharkia Governorate. All samples were investigated by California mastitis test (CMT) and bacteriological examination to determine prevalence and etiology of subclinical mastitis (SCM) in sheep and goats and to estimate the mean somatic cell count (SCC) of infected milk samples with different pathogens. The prevalence rates of SCM using CMT were 44 (40.7%) in ewes [59 milk samples, (27.3%)] and 50 (48.1%) in does [62 milk samples, (29.8%)]. Furthermore, out of ewes' and does' milk samples examined by bacteriological culture 54 (25.0%) and 56 (26.9%) samples were bacteriologically positive, respectively. The isolated bacterial causing SCM in ewes' milk samples were *E. coli* (44.4%), *S. aureus* (38.9%), *Streptococcus* spp. (27.8%), coagulase negative *Staphylococci* (26.0%), *Citrobacter* spp. (3.7%) and *Enterobacter* spp. (1.9%). The identified isolates from does' milk samples were *S. aureus* (46.4%), *E. coli* (26.8%), *Streptococcus* spp. (25.0%), coagulase negative *Staphylococci* (19.6%), *Citrobacter* spp. (5.4%) and *Enterobacter* spp. (3.6%). The geometric mean of SCC of milk samples harbored major pathogens was significantly ($P < 0.05$) higher than those harbored minor pathogens. Polymerase Chain Reaction (PCR) was applied on a total of 26 isolates from the most recovered species associated with mastitic milk samples of ewes and does. Suspected *S. aureus*, *E. coli*, *Strep. agalactiae*, *Strep. dysagalactiae*, and *Strep. uberis* isolates showed characteristic bands at 270 bp, 366 bp, 487 bp, 279 bp, and 723 bp which were specific for the used genes, respectively. In conclusion: a great attention should be directed to the early diagnosis of SCM in ewes and does.

Keywords: Ewes, Does, Mastitis, Somatic cell count, Bacteriology, PCR.

Introduction

Subclinical mastitis (SCM) is one of the most serious economic diseases of the mammary glands of goats [1] and ewes worldwide. The main reasons for its economic impact include higher prevalence rates [2] and adverse effects such as reduced milk yield in consequence with growth retardation and higher mortality rate among suckling lambs [3] and kids [4]. The disease is characterized by evolving of intramammary infection (IMI) without clinical signs [5] and is often associated with an increase in somatic cell count (SCC) [3]. The disease is usually passed undetectable and animals with SCM remain untreated.

Therefore, early detection of SCC is necessary to avoid persistent udder infection and the spread of the disease [6]. PCR assay is a reliable, accurate and confirmatory technique for the identification of pathogens especially *Staphylococcus aureus* recovered from mastitic milk samples of sheep and goats [7]. The objectives of this study were to investigate the prevalence and etiological agents of subclinical mastitis among sheep and goats in Sharkia Governorate using California mastitis test (CMT), bacteriological and molecular identification of the bacterial isolates as well as studying the relation between udder

***Corresponding author e-mail: (alshymaaamer123@gmail.com), Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt.**

infection and the level of milk somatic cell count have been carried out.

Materials and Methods

Animals

From September 2016 to August 2017, a total of 212 apparently healthy lactating small ruminants comprising 108 ewes and 104 does from small private flocks in different localities at Sharkia Governorate were selected for this study based on acceptance of the owners to participate in the study.

Milk samples

A total of 424 milk samples comprising 216 ewes' and 208 does' milk samples were collected under aseptic conditions after cleaning and disinfection of teat tips with 70% ethyl alcohol [8].

California mastitis test

Each milk sample was screened with CMT as described by Schalm *et al*. [9]. An equal volume of CMT reagent was added to 2 mL of milk samples in a black plastic cup paddle and then rotated for 10 sec. The detergent in CMT lysed somatic cells and released DNA with the formation of viscous gel. The results were interpreted according to the grade of gel formation as follows; negative (no trace), trace, +1 (weak positive), +2 (distinct positive) and +3 (strong positive).

Somatic cell count

Milk samples were examined automatically using somatic cell count MT05 apparatus [10] at Food Health Department, Animal Health Research Institute, Zagazig, Sharkia. The samples were warmed at 40ºC for 5 min and then mixed before reading the results automatically.

Bacteriological examination

Milk samples were incubated aerobically at 37ºC for 24 h and then were centrifuged at 3000 rpm for 20 min. The supernatant fluid was discarded and a sterile loopful from the sediment was streaked onto blood agar base (Oxoid, CM3) enriched with 5-10% defibrinated sheep blood and then sub-cultured on selective media for different bacteria; Mannitol Salt Agar (Oxoid, CM 85) for *Staphylococcus*, Eosine Methylene Blue Agar (EMB) (Oxoid, CM 69) for *Escherichia coli* and Edwards medium (Oxoid, CM 27) for *Streptococci* spp. In addition, the samples were also cultured on MacConkey Agar (Oxoid, CM115) for differentiation of Enterobacteriacae. All the plates were incubated aerobically at 37°C for 24–48 h and then examined for gross colony morphology, pigmentation and hemolytic characteristics. Suspected colonies were purified on nutrient agar plates and subjected to Gram staining and biochemical identification using; catalase test, coagulase test, Christie, Atkinson, Munch and Peterson test (CAMP), esculin hydrolysis test, haemolysis, indole, methyl red, Voges-Proskauer, triple sugar iron, citrate utilization tests, and sugar fermentation [11-14].

Statistical analysis

The data analysis was conducted using SPSS version 21 for windows [15]. Means and Standard Error values of variables were calculated. The Mann-Whinty U-test was used for comparison between major and minor pathogens groups in terms of the SCC values. The p-value < 0.05 was considered statistically significant.

Molecular identification of the suspected isolates

A total of 26 suspected isolates (5 *S. aureus,* 6 *E. coli* and 15 *Streptococci* isolates) recovered from milk samples of subclinically infected ewes and does were subjected to further molecular identification. Molecular identification was carried out at Food Analysis Center, Faculty of Veterinary medicine, Benha University, Egypt.

The DNA from the suspected colonies was extracted using Qiagen extraction kits (Qiagen, Germany) according to the manufacturer's guidelines. The sequences of *S. aureus* [16], *E. coli* [17] and *Streptococci* [18] specific primers are listed in Table (1).

Table 1: Oligonucleotide primers' sequences and predicted sizes of PCR products

Organisms	Genes	Oligonucleotide sequence (5′ →′3)	Product size (bp)
S. aureus	*Nuc*	F:GCGATTGATGGTGATACGGTT R:AGCCAAGCCTTGACGAACTAAAGC	270
E. coli	*alr*	F:CTGGAAGAGGCTAGCCTGGACGAG R:AAAATCGGCACCGGTGGAGCGATC	366
Strep. agalactiae	plasminogen activator A	F:ATTGATAACGACGGTGTTACTGT R:CATAGTAGCGTTCTGTAATGATGTC	487
Strep. dysagalactiae	16S rRNA	F:GTGCAACTGCATCACTATGAG R:CGTCACATGGTGGAT TTTC	279
Strep. uberis	Fibrinogen binding gene	F:TGATTCCGACTACTACGCTAGAT R:ATACTTTGAGTTTCACCGAGTTC	723

Amplification of nuc gene of S. aureus

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany) using 25 µL of PCR mixture containing 3 µL of DNA extract, 200 M of desoxynucleotide triphosphate (dNTP mixture), 1.4 U of Taq DNA polymerase (Biotools, Madrid, Spain), buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl and 3 mM MgCl2, Biotools) and 20 M of each primer (*nuc*). The amplification conditions included denaturation for 5 min at 94°C, followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec, and then a final extension at 72°C for 10 min. PCR amplified products were analyzed by 1.5% of agarose gel electrophoresis stained with ethidium bromide then visualized and captured on ultraviolet transilluminator. The samples were electrophoresed at 100 volts for one hour. A 100 bp DNA Ladder (Fermentas, Germany) was used to determine the fragment sizes. Presence of 270 bp fragments indicated the presence of *S. aureus*.

Amplification of alr gene of E. coli

The PCR reaction mix (50 µL) for each sample was consistent of 10 µL extracted DNA, 2.5 µL primers mix., 1µL deoxynucleoside triphosphate (dNTP-mix), 5µL 10x buffer, 1 µL Taq-DNA polymerase enzyme (5000 U/ml) and 30.5 µL ultra pure deionized water. The first initial cycle: 95 °C for 6 min (denaturation), 35°C for 2 min (annealing) and 72 °C for 1.5 min (extension).

The consequent 35 cycles: 95°C for 20 s (denaturation), 35 °C for 60 s (annealing) and 72 °C for 60 s (extension). A final extension at 72 °C for 5 min and then holding at 4 °C. The amplified product was analyzed on agarose gel (2% agarose and 5 µL of ethidium bromide in 1 x Tris –Acetate EDTA (TAE) buffer). The samples were electrophoresed at 100 volts for one hour, shown under ultra violet transilluminator and photographed. Visible bands of appropriate size of 366 bp were considered positive.

Amplification of GSag, GSdys and GSub genes of Streptococcus species

The multiplex PCR was performed using 25 µL reaction mix, 1x HotStarTaq Master Mix, 2.5 µL of diluted lysate, and 300 nM of each primer. The reaction was performed on a Thermal Cycler. Amplification conditions were denaturation at 95 °C for 15 min followed by 35 cycles including 94 °C for 60 s, 58 °C for 60 s and 72 °C for 60 s. The PCR reaction was terminated by a final extension at 72 °C for 10 min followed by cooling down to 4 °C. The PCR products were analyzed by electrophoresis using a 1.3% agarose gel in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH = 8.3). The samples were electrophoresed at 100 volts for one hour. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. Visible bands of appropriate size of 487 bp, 279 bp and 723 were considered positive for *Strep. agalactia*, *Strep. dysagalactia* and *Strep uberis,* respectively.

Results and Discussion

Results of Table (2) reveal that the prevalence rates of SCM on the basis of CMT were 44 (40.7%) in ewes [59 udder halves, (27.3%)] and 50 (48.1%) in does [62 udder halves, (29.8%)]. In accordance, percentages

of 43.2% in ewes and 41% in does in Sharkia Governorate, Egypt [19], 40.5% in 42 first partum Santa Ines ewes in Brazil [20] and 45.8% among goats in China [21] were previously recorded. Higher prevalence rates of 66.9% in Awassi sheep in Jordan [22] and 61% among goats in Kenya [23] were previously reported. In contrary, lower prevalence rates of 6.9% among sheep and 14% among goats were recorded in El-Behera Governorate, Egypt [7]. The differences in the managemental practices, geographical distribution, health status of the flock, weather, nutritional status and finally the size of the study samples may be the reason for the variation in the prevalence rates between the present study and the previous ones

Table 2: Prevalence rate of subclinical mastitis based on California Mastitis test

Animal species	No. of examined animals	Positive cases		No. of examined halves	Positive CMT	
		No.	%		No.	%
Ewes	108	44	40.7	216	59	27.3
Does	104	50	48.1	208	62	29.8

Table 3: Prevalence rate of subclinical mastitis in ewes' and does' milk samples based on the results of bacteriological examination

Animal species	No. of examined milk samples	Positive samples	
		No.	%
Ewes	216	54	25
Does	208	56	26.9
Total	424	110	25.9

Results in Table (3) show that the prevalence rates of bacteriologically positive milk samples of subclinically mastitic ewes and does were 25% and 26.9%, respectively. Higher prevalence rates of 43.3% in ewes and 58.5% in does were reported in Sharkia Governorate, Egypt [19]. However, lower prevalence rates of 8.08% in ewes and 15.2% in does were recorded in Assiut Governorate, Egypt [24].

Results in Table (4) illustrate that the most prevalent bacterial species isolated from milk samples of ewes was *E. coli* (44.4%) followed by *S. aureus* (38.9%). Lower isolation rates of *E. coli* from milk samples of subclinically infected sheep were 11.3% in Turkey [25] and 19.6% in Jordan [26]. *S. aureus* was isolated from subclinically mastitic milk samples of sheep with the percentages of 23.1 in Egypt [7] and 39 in Jordan [26].

Concerning goats' milk samples, the results reveal that *S. aureus* represents the most predominate isolated bacterial species (46.4%) followed by *E. coli* (26.8%). These results are in agreement with those previously recorded in Sharkia Governorate, Egypt [27] where the main isolates recovered from subclinically mastitic goats' milk samples were *S. aureus* (40%) followed by *E. coli* (26.67%). Lower isolation rates of 23.4% for *S. aureus* and 21.3% for *E. coli* were reported in Ethiopia [28]. *S. aureus* and *E. coli* were respectively recovered from 69.6% and 30.23% of goats' milk in Turkey [29] and Assiut Governorate, Egypt [30]. The highest isolation rate of *E. coli* as environmental pathogen recovered from milk samples of subclinically infected ewes may be attributed to managemental mistakes as overcrowding, bad ventilation, inadequate manure removal and general lack of farm cleanliness and sanitation as recorded in Jordan [22].

Table 4: Frequency of microorganisms isolated from milk samples positive for mastitis in ewes and does

Bacterial isolates	Ewes		Does	
	No.	**%***	**No.**	**%***
Staphylococcus aureus	21	38.9	26	46.4
Escherichia coli	24	44.4	15	26.8
Streptococcus spp	15	27.8	14	25.0
Coagulase negative *Staphylococci*	14	26.0	11	19.6
Citrobacter spp.	2	3.7	3	5.4
Enterobacter spp.	1	1.9	2	3.6

*in relation to bacteriologically positive milk samples

Table 5: Relation between Somatic Cell Count (× 10^3 cell/ml) and etiological agent isolated from milk of subclinically infected animals

Pathogens	Ewes			Does		
	No.	**%****	**SCC (GM± SEM)**	**No.**	**%****	**SCC (GM± SEM)**
Major pathogens:	28	51.9	410.6±53.4	34	60.7	471.5±54.2
S. aureus	10	18.5	504.9±115.9	19	33.9	569.4±82.9
E. coli	10	18.5	415.6±175.3	5	8.9	470.8±148.4
Streptococcus spp.	8	14.8	312.3±44.5	10	16.1	329.8±48.2
Minor pathogens:						
Coagulase negative Staphylococci	9	16.7	236.9±26.4	7	12.5	251.2±43.3
Mixed infection	17	31.9	645.6±74.9	15	26.8	608.5±108.8
Total	54		431.9±38.7	56		466.7±45.3

** % of infected udder halves.
GM: geometric mean, SEM: Standard error of mean.
Significant difference between SCC of major pathogens and minor pathogens (P value < 0.05).

In the current study, *Streptococci* species were recovered from 27.8% of ewes' positive samples. This result is in contrary with numerous studies which reported lower isolation rates of 7.4% [7] and 15.2% [30] in Egypt and 12% in Brazil [31].

In goats, *Streptococci* were isolated from 25% of positive samples. This rate was higher than that previously recorded in Egypt [30] and lower than that (56%) stated in Botswana [32].

Coagulase negative *Staphylococci* in the present study represent the lower isolation rate than *pathogens. aureus*, *E. coli* and *Streptococci*. It represents 26% and 19.6% of ewes and does positive samples, respectively. These results are in consistence with several studies which recorded that the most prevalent pathogens of the mammary gland in subclinical mastitic sheep and goats were Coagulase negative *Staphylococci* and its isolation rate ranges from 45% to 48% ovine positive milk samples as recorded in Poland [1] and USA [33], respectively. However, it ranges from 60% to 80.7% in caprine milk samples in Poland and United Kingdom [1,34].

The least identified pathogens from ewes and does positive samples were *Citrobacter* species and *Enterobacter*. Their isolation rates were (3.7% Vs 1.9%) in ewes' positive samples and (5.4% Vs 3.6%) in does' positive samples. In addition, *Enterococus fecalis* was recovered from one doe milk sample (1.8%).

In Egypt, *Enterobacter* species were previously recovered from 1.5% and 4.06% of subclinically mastitic milk of sheep and goats, respectively [30].

The intensity of cellular immune defense could be indicated by Somatic cell count and it represents a marker of the sanitary status of the mammary gland as it increases during the course of intramammary infection as a result of migration of leukocytes from the blood towards the mammary gland [35]. As shown in

Table (5) the results reveal that the mean Somatic Cell Count in milk samples of subclinically infected ewes with major and minor pathogens was 410.6 ± 53.4×10^3 and 236.9 ± 26.4×10^3 cell/mL, respectively. While, in goats it was 471.5 ± 54.2×10^3 and 251.2 ± 43.3 × 10^3 cell/mL, in those infected with major and minor pathogens, respectively. These results showed that the mean SCC of the udder halves infected with major pathogens was significantly ($P < 0.05$) higher than those infected with minor pathogens. This finding is consistent with Alekish *et al*. [22] in Jordan who found that most of the milk samples of subclinically infected ewes with *S. aureus* as a major pathogen had SCC more than 1×10^6 cell/mL. While, coagulase negative staphylococci as minor pathogens showed moderate increase in SCC (250×10^3- 1×10^6 cell mL). In goats, Anniss and McDougall [36] found that the SCC of the glands infected with a major pathogen was significantly ($P < 0.05$) higher than glands with a minor pathogen infection (800×10^3 and 481×10^3 cell/mL), respectively. The higher SCC of milk samples from animals infected with a major pathogen might be attributed to its pathogenicity that triggers the immune system. Coagulase negative *Staphylococci* are less pathogenic for the mammary gland of domestic ruminants; therefore, they produce persistent subclinical mastitis with less stimulation of immune system and then modest increase in SCC. Hence, the increase in the SCC might depend on the host immune system at the time of the infection in addition to the pathogenicity of the isolated organism [22]. Knowing such correlation between the SCC and the isolates is necessary for predicting the target pathogen when screening for the presence of mastitis and planning a control program.

The results of the molecular identification of the suspected isolates reveal that the investigated specific gene for each pathogen was confirmed in all 26 isolates examined. Thus, indicating the high specificity and sensitivity of PCR assay in the detection of pathogens.

Conclusion

The higher prevalence of subclinical mastitis among small ruminants alarming that attention should be directed toward such form of mastitis, Somatic cell count and intramammary infection are significantly associated with bacterial isolates, *S. aureus* and *E. coli* are identified as the predominant etiologic agents of SCM among small ruminants.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Olechnowicz, J. and Jaskowski, J.M. (2014): Mastitis in small ruminants. Med Weter, 70 (2): 67-72.

[2] El-Khabaz, K.A.S.; Malek, S.S. and Hussein, H.A. (2015): Bacteriological, cytological, and hematological changes associated the bovine subclinical mastitis. Assiut Vet Med J, 61 (145): 236-241.

[3] Ebrahimi, A.; Lotfalian, Sh. and Karimi, S. (2007): Drug resistance in isolated bacteria from milk of sheep and goats with subclinical mastitis in Shahrekord district. Iranian Journal of Veterinary Research, University of Shiraz, 8 (1): 76-79.

[4] Kumar, R.; Gupta, D.K.; Bansal, B.K.; Singh, S.; Sharma, S.; Kumar, A. and Uppal, S.K. (2016): Prevalence, current antibiogram and risk factors associated with mastitis in dairy goats in Punjab. International Journal of Science, Environment and Technology, 5 (6): 4580-4593.

[5] Marogna, G.; Pilo, C.; Vidili, A.; Tola, S.; Schianchi, G. and Leori, S.G. (2012): Comparison of clinical findings, microbiological results and farming parameters in goat herds affected by recurrent infectious mastitis. Small Ruminant Research, Amsterdam, 102 (1): 74-83.

[6] Siugzdaite, J.; Gabinaitiene, A. and Siugzda, R. (2016): Antimicrobial resistance of pathogens from ewes' subclinical mastitis. Vet Med Zoot., 73 (95): 122-127.

[7] Akram, A.G.E. (2015): Clinical, epidemiological and bacteriological studies on mastitis in small ruminant. M.V.Sc., Thesis (Infectious diseases), Faculty of

Veterinary Medicine, Alexandria University.

[8] Dego, O. K., and Tareke, F. (2003): Bovine mastitis in selected areas of southern Ethiopia. Tropical animal health and production, 35 (3): 197-205.

[9] Schalm, O.W.; Carroll, E.J. and Jain, N.C. (1971): Bovine Mastitis. Philadelphia: Lea & Febiger London: Baillière, Tindall & Cassell.

[10] Constable, P. D.; Hinchcliff, K. W.; Done, S. H. and Grünberg, W. (2017). Neonatal infection disease. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. 11th edition. Elsevier, China. p1874-1903

[11] Quinn, P.; Markey, B.; Carter, M.; Donnelly, W. and Leonard, F. (2002): Veterinary Microbiology and Microbial Disease. Black Well Science, pp. 121.

[12] Cruickshank, R.; Duguid, J.P.; Marinion, B.P. and Swain, R.H. (1975): Textbook of Medical Microbiology 12 th edition. Vol. II. Churchill, Edinberg. pp. 343.

[13] Koneman, W.E.; Allen, S.D.; Danda, M.W.; Schreckenberger, P.C. and Win, W.C. (1997): Color Atlas and Textbook of Diagnostic Microbiology. 5th edition, Lippincott, Philadelphia, New York.

[14] Krieg, N. and Holt, J. (1984): Bergey's Manual of Systemic Bacteriology. Vol. 1. William and Wilkins, Baltimore, M.D.21202, USA.

[15] SPPS, I. (2012). IBM SPSS statistics version 21. Boston, Mass: International Business Machines Corp, 126.

[16] Brakstad, O. G; Aasbakk, K. S. and Maeland, J. A. (1992**):** Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of nuc gene for enterotoxins. J. Clin. Microbiol., 30 (7): 1654-1660.

[17] Yokoigawa, K.; Inoue, K.; Okubo, Y. and Kawai, H. (1999): Primers for amplifying an alanine racemase gene fragment to detect *E. coli* strains in foods. J. Food Sci., 64 (4): 571–575.

[18] Raemy, A.; Meylan, M.; Casati, S.; Gaia, V.; Berchtold, B.; Boss, R.; Wyder, A. and Graber, H.U. (2013): Phenotypic and genotypic identification of streptococci and related bacteria isolated from bovine intramammary infections. Acta Veterinaria Scandinavica, 55 (1): 53-61.

[19] El-mesalami M.M.Y. (2010): Molecular technique using for diagnosis of Mycoplasma and some bacteria in mastitic sheep and goats. M.V.Sc., Thesis (Bacteriology), Faculty of Veterinary Medicine, Zagazig University.

[20] Oliveira, A.A.; Melo, C.B.; Seixas, L.; Azevedo, H.C.; Teixeira, K.M.; Melo, P.O.; Emidio, K.S.; Oliveira, S.S. and McManus, C. (2013): Mastitis and milk composition in first partum Santa Ines ewes. J. Vet. Adv., 3 (8): 220-231.

[21] Zhao, Y.; Liu, H.; Zhao, X.; Gao, Y.; Zhang, M. and Chen, D. (2015): Prevalence and pathogens of subclinical mastitis in dairy goats in China. Trop. Anim. Health Prod., (47):429-435.

[22] Alekish, M.O.; Alshehabat, M.A. and Abutarbush, S.M. (2014): The prevalence and etiology of subclinical mastitis in Awassi sheep; emphasis on the relationship between the isolated organisms and the somatic cell count. European Journal of Veterinary Medicine, 8: 1-13.

[23] Mbindyo, C.M.; Gitao, C.G. and Bebora, L. (2014): A cross-sectional study on the prevalence of subclinical mastitis and antimicrobial susceptibility patterns of the bacterial isolates in milk samples of smallholder dairy goats in Kenya. American Journal of Research Communication, 2 (8): 30-51.

[24] El-Bassiony, T.; El-Prince, E.; Abd El-Hameed, K.G.; Abd El-Haleem, A.A. and Sadek, O.A. (2008): Prevalence and public health hazard of subclinical mastitis in goats and sheep in Assiut Governorate. Assiut Vet. Med. J., 54 (118): 108-117.

[25] Ozenc, E.; Seker, E.; Baki Acar, D.; Birdane, M.K.; Darbaz, I. and Dogan, N. (2011): The importance of Staphylococci and threshold value of somatic cell count

for diagnosis of sub-clinical mastitis in Pirlak sheep at mid-lactation. Reprod Dom Anim., 46 (6): 970-974.

[26] Al-Majali, A.M. and Jawabreh, S. (2003): Period prevalence and etiology of subclinical mastitis in Awassi sheep in southern Jordan. Small Rum. Res., 47 (3): 243-248.

[27] Sameer, M.M.; El-Kabbany, M.F.; Abeer, M.A. and Eid, S. (2013): Inflammatory markers as laboratory tool for early diagnosis of subclinical mastitis in goats with special reference to their effect on the milk quality. Zag. Vet. J., 41 (4): 171-178.

[28] Haftay, A.; Habtamu, T.M. and Abebe, M.S. (2016): Bacterial identification and antimicrobial susceptibility of subclinical mastitis causing bacteria from goats in Aba'lla district, Afar, North-Eastern Ethiopia. Revue Med. Vet., 167 (7-8): 170-175.

[29] Isnel, N.B. and Kırkan, S. (2013): Isolation of microorganisms from goats with subclinical mastitis and detection of antibiotics susceptibility. Animal Health, Prod. and Hyg., 1(2): 106-112.

[30] Sedel, S.M.; Al-Habaty, S.H. and Makar, N.H. (2012): Bacteriological studies on subclinical mastitis of small ruminants in Assiut Governorate. Egypt. J. Agric. Res., 90 (1): 117-129.

[31] Santana, R.C.M.; Zafalon, L.F.; Esteves, S.N.; Tanaka, E.V.; Pilon, L.E. and Massa, R. (2013): Occurrence of etiological agents causing subclinical mastitis in Morada Nova and Santa Ines ewes. Ars Veterinaria, 29 (3): 148-152.

[32] Mugabe, W.; Nsoso, S.J.; Mpapho, G.S.; Kamau, J.M.; Mahabile, W.; Shah, A.A.; Nazar, M.; Khan, I.U.; Kaka, N.A. and Shah, I.A. (2017): Occurrence of caprine mastitis and its etiological agents and associated selected risk in mid lactating goats in the Oodi extension area of Kgatleng District, Botswana. Academic Web Journal of Agricultural Research, 2 (1): 014-020.

[33] Spanu, C.; Bergre, Y.M.; Thomas, D.L. and Ruegg, P.L. (2011): Impact of intramammary antimicrobial dry treatment and teat sanitation on somatic cell count and intramammary infection in dairy ewes. Small Ruminant Res., 97:139-145.

[34] Hall, S.M. and Rycroft, A.N. (2007): Causative organisms and somatic cell counts in subclinical intramammary infections in milking goats in the U.K. The Vet. Rec., 160 (1): 19-22.

[35] Gonzalo, C.; Ariznabarreta, A.; Carriedo, J.A. and San Primitivo, F. (2002): Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes. J. Dairy Sci., 85 (6): 1460-1467.

[36] Anniss, F. and McDougall, S. (2000): Prevalence of bacterial infection and somatic cell count in early postpartum milking goats. In Proceedings of the New Zealand Society of Animal Production, 60: 284-286.

الملخص العربي

معدل الاصابة ومسببات التهاب الضرع الكامن في الاغنام والماعز

[1]الشيماء عبد الفتاح عبد الله ,[2]محمد إبراهيم عيسى و [2]عفاف محمد منازع
[1] المستشفى البيطرى - كلية الطب البيطرى - جامعة الزقازيق - مصر
[2]قسم طب الحيوان - كلية الطب البيطرى - جامعة الزقازيق – مصر

تم تجميع 424 عينة لبن من أنصاف ضروع 212 حيوان حلاب غير مصاب ظاهريا (108 نعاج و 104 ماعز) في الفترة من سبتمبر 2016 الي اغسطس 2017 من القطعان الصغيرة من أماكن مختلفة بمحافظة الشرقية وتم فحص هذه العينات باختبار الكاليفورنيا بالاضافة الي الفحص الميكروبيولوجى وذلك لتحديد مدى إنتشار التهاب الضرع الكامن فى النعاج والماعز وأهم مسبباته. وأيضا تحديد عدد الخلايا الجسدية فى العينات المصابة بالميكروبات المختلفة. بفحص عينات الألبان لكل من الأغنام والماعز باختبار الكاليفورنيا أسفرت النتائج أن 59 (27.3٪) و 62(29.8٪) كانت إيجابية ، أما بالنسبة لفحص الحيوانات فقد كانت النتائج إيجابية بنسبة 44 (40.7٪) و50 (48.1%) لكل من النعاج والماعز على التوالى. كما كانت نتائج الفحص البكتريولوجى لعدد 216 و 208 عينة لبن لكل من النعاج والماعز إيجابية بنسبة 54 (25٪) و56 (26.9٪) لكل منهما على التوالى . كما أظهرت النتائج أن البكتيريا المعزولة و المسببة لالتهاب الضرع الكامن فى النعاج هى الميكروب القولونى (44.4٪)، المكور العنقودى الذهبى (38.9٪)، المكورات السبحية (27.8٪)، المكور العنقودى السالب التجلط (26٪)، السيتروباكتر (3.7٪) والانتيروباكتر (1.9٪). بينما فى الماعز كان المكور العنقودى الذهبى (46.4٪)، الميكروب القولونى (26.8٪)، المكورات السبحية (25٪)، المكور العنقودى السالب التجلط (19.6٪)، السيتروباكتر (5.4٪) ،الانتيروباكتر (3.6٪). وفيما يختص بعدد الخلايا الجسدية, أوضحت النتائج أن عددها فى العينات المصابة بالميكروبات العالية الضراوة أعلي معنويا منها في العينات المصابة بالميكروبات الأقل ضراوة. باجراء اختبار تفاعل البلمرة المتسلسل علي 26 عترة من العترات المعزولة من لبن النعاج والماعز المصابة بالتهاب الضرع الكامن تم التأكد من العزلات المختبرة (المكور العنقودي الذهبي, الميكروب القولونى، المكور السبحى اجالاكتيا، المكور السبحى ديس اجالاكتيا والمكور السبحى يوبريس) وذلك لوجود منطقة مميزة للعترات المختبرة مقارنة بالعترات المرجعية عند الوزن الجزيئي 270, 366, 487, 279و723 قاعدة مزدوجة علي التوالي. وتوصي هذه الدراسة بضرورة توجيه الاهتمام بالتشخيص المبكر لالتهاب الضرع الكامن فهو يعتبر مصدر لانتشار العدوي بالاضافة الي تفادى تطوره لالتهاب الضرع الاكلينيكى.