

Trials To Control of Mastitis in goats By Using Biological Bacterial Product As An Immunostimulant

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ABSTRACT

Mastitis is recognized as one of the most important diseases affecting dairy animals resulting in substantial economic losses worldwide due to reduced milk production and quality. The present study was designed to evaluate the effect of antibiotic treatment and Priplasmic membrane protein (PMP) on mastitis and immunomodulating effect in mastitic dairy goats.

The experiment was carried out on 10 Lactating goats suffered from mastitis that were divided into two groups .1st group was treated by antibiotic of choice only and the 2nd treated by antibiotic and Priplasmic membrane protein (PMP).

A number of 10 milk samples were collected from examined mastitic dairy goats and examined for SCC, TBC, isolates identification, antibiotic sensitivity test Nitric oxide level and Lysozyme level before & after treatment).Coagulase negative staph, *S. aureus*, Coliform & *St.dysagalactia* were isolated in percentage 100%,80%,70% and 50% respectively. Amoxicillin + Clavulanic Acid is the drug of the choice according to sensitivity test.

After treatment by antibiotic and PMP.TBC & SCC were decreased more than the treatment by antibiotic only, Meanwhile on the other side immunostimulating parameter (Nitric oxide & Lysozyme) were increased .

So the current work point out that the concurrent administration of biological preparation (PMP) with therapeutic drug (Synulox®) improve the immune response (nitric oxide and lysozyme activity) in treated goats.

INTRODUCTION

Mastitis is defined as an inflammatory reaction of the mammary gland (1). It is induced when pathogenic microorganisms enter the udder through the teat canal, overcome the animal's defense mechanisms, begin to multiply in the udder, and produce toxins that are harmful to the mammary gland. A major economic loss of mastitis is decreased milk production as a result of udder damage. Mastitis in ewes (based on California Mastitis Test scores) has been shown to reduce milk production by 12% and 58% with one or both udder halves infected, respectively.

Mastitis is a multifactorial disease. as such, its incidence depends on exposure to pathogens, effectiveness of udder defense mechanisms, and presence of environmental

risk factors, as well as interactions between these factors (2,3). It can be caused by physical injury or stress or by bacteria which invade the mammary gland. The bacteria which are known to cause mastitis in cows, sheep and goats are *Streptococcus sp.*, *Staphylococcus sp.*, *Pasteurella sp.*, and coliforms, such as *E. coli*.

Treatment of mastitis with antibiotics is not an ideal solution. Other than the problems they cause with the milk (withdrawal for x days, contamination from antibiotic residues, problems associated with yogurt and cheese processing), antibiotics have not reduced the incidence of mastitis (4). Problems associated with resistance or even ineffectiveness are quite real in the case of mastitis caused by coliforms and *Staphylococcus aureus* (5). Most treatments are given intramuscular (IM),Intramammary

treatments have been used in dairy cattle, but sheep data is limited.

Stemmed from the previous notion, this study was designed to:

- 1- Examine the immunomodulating effects of antibiotic used for mastitis treatment as the drug of choice and biological preparation "periplasmic membrane proteins (PMP) extracted from *E. coli* on the immunological response naturally mastitic dairy goats .
- 2- To evaluate the benefit income on the immune response by using of both of them together compared with using antibiotic as a single drug for treatment (6)

MATERIAL AND METHOD

Experimental Animal

This study was carried out on dairy goats farm of Animal Reproduction Research Institute on 10 lactating goats suffered from mastitis and were divided into two groups .

1st group was treated by antibiotic of choice only.

2nd treated by antibiotic and periplasmic membrane proteins (PMP).

Milk Samples

A Milk samples were collected from examined goats before and after treatment. Each milk sample was collected in clean, sterile and dry McCartney glass in duplicate. One sample was examined for California mastitis test (C. M.T) and somatic cell count (S.C.C.) while the other was used in bacteriological studies.

Field Test (California Mastitis Test, (CMT)

According to APHA (1992) for detection of subclinical mastitis in goats, depending on the amount of gel formation, all samples were assigned to 3 categories: negative or positive reaction in 2 grades (++ and +++).

Measurements of somatic cell count (S.C.C)

It was estimated automatically using SOMA-COUNT 150, from Bentley .The SCC measures the number of white blood cells and udder squamous epithelial cells in milk that were present in large number in case of mastitis.

Bacteriological examination

All milk samples were collected from infected goats for bacteriological examination:

- 1- Total bacterial counts.
- 2- Culturing on different specific media (sheep blood media, Manitol salt agar, Edward's media MacConky agar media).

Isolation and identification of causative organisms

Isolation and identification of causative organisms were done according to (7).

Antibiotic sensitivity test

The used antibiotic discs consisted of Erythromycin, Oxytetracyclin, Gentamycin, Amoxicillin, Amoxicillin with Clavulanic Acid, Streptomycin, Rifampicin and Neomycin discs were manufactured by Pasture Lab, Egypt. The test was done according to National Committee for Clinical Lab Standard (8).

Peri-plasmic membrane protein (PMP)

Extraction of Peri-plasmic protein from *E.coli* was made according to (9).

Measurement of Nitric oxide production by macrophage as described by (10).

Measurement of lysozyme activity by using agarose gel cell lysis assay according to the method described by (11.)

Treatment

The infected goats were divided into two groups.

The 1st group was clinically treated using only Synulox® (amoxicillin with clavulanic acid) according to the result of antibiotic sensitivity test using the recommended dose (8.75mg/kg body weight where 7.0 mg amoxicillin, 1.75mg clavulanic acid) i.e

1ml/20kg body weight via intra muscular injection .

The 2nd group was injected by Synulox® and PMP in a dose of 40_{mg}/animal

antibiotic treatment evoked significance reduction in total bacterial count and somatic cell count in milk of treated dairy goats.

Meanwhile, the reduction in total bacterial count and somatic cell count in group 2nd. Group produce significance reduction in total bacterial count and somatic cell count in milk in compare with antibiotic only treated group.

RESULT

In table (1) antibiotic treatment induce significance reduction in total bacterial count and somatic cell count in milk in compare with self control of the group. Also, preplasmic &

Table 1.Total Bacterial Count (T.B.C) & Somatic cell Count (SCC) in Mastitic Dairy goats.

	Before		After	
	Mean TBC ±S.E	Mean SCC ± S.E	Mean TBC ± S.E	Mean SCC ± S.E
1 st (Antibiotic)	166 ± 34 ^{Aa}	756 ± 61 ^{Cc}	36 ± 4 ^{Bb*}	235 ± 23 ^{Dd*}
2 nd (Antibiotic & preplasmic protein)	165 ± 24 ^{Aa}	724 ± 47 ^{Cc}	22 ± 3 ^{Fe*}	148 ± 22 ^{Hh*}

Means carrying different superscripts (small letters) within rows are significant at p < 0.05

Means carrying different superscripts (capital letters) within columns are significant at p < 0.05

Table 2. reveals that coagulase negative *Strept dysagalactia* is the most common *Staphylococci* , *Staph aureus* , *Coliform* and isolates in goats milk.

Table 2. Prevalence of M.O in cases of subclinical mastitis in ewes milk.

Bacterial Isolates	No. Isolates	Prevalence
Coagulase negative staph (CNS)	10	100%
<i>S.aureus</i>	8	80%
<i>Coliform</i>	7	70%
<i>St.dysgalactia</i>	5	50%

Table 3. Show that antibiotic treatment did not induce any significant change in Nitric oxide level throughout the duration of experiment. Meanwhile antibiotic & PMP administration induce significant increase in 3 days Nitric oxide level in compare with the data recorded before treatment.

Group	Before	After 1 day	After 2 days	After 3 days
1 st (Antibiotic)	13.5 ^a ±0.53	11.51 ^{aB} ±3.8	15.95 ^{aB} ±0.43	24.19 ^{bB} ±2.18
2 nd (Antibiotic & preplasmic protein)	15.19 ^{aB} ±1.94	26.91 ^{bC} ±2.3	65.1 ^{cC} ±1.75	153.1 ^{dD} ±13.5

Means carrying different superscripts (small litters) within rows are significant at p < 0.05

Means Carrying different superscripts (capital litters) within columns are significant at p < 0.05

Table 4. The single treatment with antibiotic in lysozyme level. Meanwhile simultaneous first group did not elucidate any significant change administration of antibiotic & PMP. Induce

significant increase in Lysozyme activity. also antibiotic & PMP second group Shaw significant increase in Lysozyme activity in compare with antibiotic treated group.

Table 4. The effects of Syanluox® and periplasmic membrane (PMP) on Lysozyme production in mastitic Dairy goats.

	Before	After 1 day	After 2 days	After 3 days
1st. (Antibiotic)	192.1 ^{ab} ±17.6	181.2 ^{ab} ±16.8	178.1 ^{ab} ±10.9	193.5 ^{ab} ±18.9
2nd. (Antibiotic & preplasmic protein)	190.3 ^{ab} ±2.03	210.18 ^{bc} ±4.93	291.13 ^{cd} ±13.78	336.3 ^{cd} ±13.5

Means carrying different superscripts (small litters) within rows are significant at $p < 0.05$

Means carrying different superscripts (capital litters) within columns are significant at $p < 0.05$.

DISCUSSION

In last years, the dairy goat industry is rapidly gaining in importance throughout the world. Therefore, any factor that adversely affects the quantity and quality of goat milk is of great financial interest.

Mastitis is a significant problem in dairy goats flocks and leads to decreased milk production. Mastitis has importance for 3 perspectives: economic, hygienic, and legal (12).

The prevalence of subclinical sheep mastitis ranges between 7.05% and 92% and it occurs worldwide (13).

The present study reveals that *Staphylococci* (CNS100% & *S.aureus* 80%), Coliform 70% and *Strept dysagalactia* 50% are the most common isolates in mastitic dairy goat milk. on the similar ground, the isolated strains shows sensitivity towards Amoxicillin-Clavulanic Acid, Oxytetracycline and Erythromycin in ratio of 100%, 90% and 90% respectively.

The obtained data on the same line of the finding of (14), they isolate *S. aureus* (69.6%), CNS. (18.6%)

and *E. coli* (20.0%). As a result of antibiotic susceptibility tests, *S. aureus* isolates were found susceptible to Amoxicillin- Clavulanic Acid in the ratio of 100%, susceptible to Penicillin in the ratio of 100%, other *Staphylococci* isolates were found susceptible to Amoxycillin- Clavulanic Acid in the ratio of

100% and *E. coli* susceptible to Ampicillin in the ratio of 100% and Amoxicillin- Clavulanic Acid in the ratio of 90% .

Also our findings are agree with the data obtained by (15) where they found 1Coagulase-negative staphylococci (CNS) were the most frequently isolated and most widespread microorganisms in the sample and they were present in all flocks (100%).

The most effective antibiotics were Cephalothin (97.4%), Sulfamethoxazole-trimethoprim (97.4%), Amoxicillin + Clavulanic Acid (97.4%) .Streptococci were isolated in 43.8% of the flocks.

The recorded data show that antibiotic (Synulox®) induce non significant change in nitric oxide after 2 days of administration, then elevated significantly after 3days . Meanwhile on the lysozyme production level the antibiotic did not elucidate any significant change.

The obtained data supported with the data recorded by (16); Who reported that the use of amoxicillin and clavulanic acid cause immune suppression symptoms in human in the form of diarrhea and candidiasis. Also (17). where found that blood born *staphylococcal aureus* pneumonia are associated with amoxicillin administration in children and (18) declare that Clavulanic and Amoxicillin cause hepatotoxic and immunosuppressive symptoms in female patient.

In the last 25 years, antibiotic modulation of the host-pathogen interaction has become the subject of increased investigational activity,

most of these studies proved the immunosuppressive effects of antibiotics on the host defense mechanism (19).

On the other hand, there is a concept that there is an immunostimulating activity of the bacterial cell wall preparations; this effect seems to involve activation of the complement system and the release of the fragments from the complement factors which are mediators of the immune response (6)

The obtained data show that antibiotic & periplasmic (PMP) administration augment lysozyme and nitric oxide level significantly in compare with antibiotic treated group.

The current work it has been pointed out that the concurrent administration of biological preparation (PMP) with therapeutic drug (Synulox®) improve the immune response (nitric oxide and lysozyme activity) in treated goats. Defiantly, The obtained data bear more over, a heavy resemblance to those validated by (20) they recorded that the immunostimulant activity of the bacterial cell wall preparation and they suggested that, this effects may be due to activation of the complement system and release fragment from complement factors which are mediator of immune response.

In the current hypothesized that, PMP of *E. coli* may interact with periplasmic phase of inner membrane via C-terminal amphiphilic alfa-helices, and theoretical analysis had predicted that. this C-terminal helical regions may be membrane interaction in immune response process (21) it seems essential, from this prospective to emphasis that, these immunotherapy dose not involve recognition of a specific antigen or to regulate the immune response toward specific pathogen but it causes an over all immune response that hastens recognition of foreign protein, restimulate the normal immune response. After a period of immune suppression (22), in this context, it is a kin to mention that, the proteins of the cell wall of the organisms is a potent immunogen (23) and the bacterial cell wall proteins are strong immune-potentiator that activate early innate host responses and cell like macrophages and epithelial cells. In addition,

they are potent polyclonal activators of murine B-lymphocytes and they also initiate the adjuvant effects on the adaptive immune system (24).

Taken together, are finding highlight the fact that the immunosuppressive effect of Amoxicillin + Clavulanic Acid could preclude their high efficacy. Nevertheless the current study advocates its concurrent administration with periplasmic membrane proteins (PMP) extracted from *E. coli* to potentiate the immune response in the mastitic dairy goat.

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composition the cell wall and cytoplasmic

الملخص العربي

محاولات للسيطرة على التهاب الضرع في الماعز باستخدام منتج بكتيري حيوى كمنشط مناعى

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**قسم الضرع و النتاج معهد بحوث التناسليات

يعد التهاب الضرع فى الماعز من أهم واطغرا لأمراض التى تؤدى إلى خسارة اقتصادية كبيرة تتمثل فى قلة انتاج وجودة اللبن وقد اجريت هذه الدراسة لتقييم أداء منتج بكتيرى حيوى مستخلص من الايشريشياكولاي (بروتينات الغلاف البلازمى) كمحفز ومنشط مناعى يستخدم مع المضاد الحيوى لتنشيط الجهاز المناعى للحيوان أثناء فترة العلاج وقد اجريت التجربة على عدد ١٠ حيوانات من الماعز الحلابه المصابة بالتهاب الضرع حيث اجري لهم اختبار كاليفورنيا وكانت النتيجة ايجابية. تم أخذ عينات لبن من الحيوانات حيث تم عد الخلايا الجسيمية والعد البكتيرى الكلى وعزل وتصنيف المعزولات البكتيرية ، كما تم إجراء اختبار الحساسية للميكروبات المعزولة و قياس نسبة أكسيد النيتريك والليزوسيم واعتبار هذه العينات مجموعة ضابطة ذاتية. ثم قسمت الحيوانات الى مجموعتين : تم اعطاء المجموعة الأولى المضاد الحيوى فقط حسب نتائج اختبار الحساسية (أموكسيسيللين + حمض الكلفيلينيك) بينما تم اعطاء المجموعة الثانية المضاد الحيوى + بروتينات الغلاف البلازمى . وتم أخذ عينات اللبن لإجراء اختبارات العد البكتيرى الكلى ، عد الخلايا الجسيمية ، قياس نسبة أكسيد النيتريك ونشاط الليزوسيم .

وقد أوضحت النتائج : عزت كل من الميكروبات المكور العقودى، المكور العقودى الذهبى، الكوليفورم والمكور السبحى ديس اجالاكتيا بنسبة (١٠٠% ، ٨٠% ، ٧٠% ، ٥٠%) على التوالى. وكانت هذه النتائج حساسة للمضاد الحيوى سينالوكس (أموكسيسيللين + حمض الكلفيلينيك) بنسبة ١٠٠%.

أظهرت النتائج ان المجموعة الثانية (المعالجة بالمضاد الحيوى + المستخلص البكتيرى) أكثر ايجابية حيث انخفضت نسبة العد البكتيرى الكلى، عد الخلايا الجسيمية انخفاضاً معنوياً بالمقارنة بالمجموعة الأولى كما سجلت النتائج زيادة معنوية فى معدل إنتاج أكسيد النيتريك وكذلك معدل نشاط الليزوسومات فى المجموعة الثانية بالمقارنة بالمجموعة لأولى

وحيث انه لان علاج الماعز والاعنام من التهاب الضرع محدود للغاية ولا توجد محاقن للضرع خاصة بهم فانه ينصح باعطاء المنتج البكتيرى الحيوى (بروتينات الغلاف البلازمى) عند اعطاء المضاد الحيوى عن طريق الحقن لتنشيط الجهاز المناعى وتقوية ذلك للعمل على زيادة وسرعة استجابة الحيوانات للعلاج