j.Egypt.net.med.Assoc 78, no 1. 121 – 134 (2018)

EFFICACY OF A POLYVALENT MASTITIS VACCINE AGAINST UDDER INFECTION WITH *STAPHYLOCOCCUS AUREUS* IN DAIRY COWS IN EGYPT

By

Eisa, A.M. A[1]; Abo El-Hassan, D.G.[2] and Samir, A.[2]

[1] General Organization for Veterinary Services, [2]Faculty of Veterinary Medicine-Cairo University

ABSTRACT

Staphylococcus aureus is a common udder pathogen in dairy cows, and cause severe mastitis problems in some herds in Egypt. In herds where normal control measures are not successful, vaccination might be an additional tool to use if sufficiently efficient. In the last years the knowledges on cow mastitis are remarkably improving, nevertheless the attention has been never focused on vaccination as preventive strategy for the control of mastitis. A, successful Staphylococcus aureus vaccine should elicit a long-term antibody response that prevents establishment of the infection. As sera of immunized pregnant heifers with *S. aureus* lysed cells formulated with a classical adjuvant stimulate antibodies production that inhibited internalization in mammary epithelial cells (MEC) and increased phagocytosis by milk macrophages, the aim of the present study was to evaluate the humoral immunological response to a commercially available vaccine. (Lysigin™, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) and its potential use as a preventive way for *S. aureus* mastitis in Holstein cows Vaccinated (V) and not vaccinated (N-V) groups, of 30 cows, were selected from one herd of the same farm. The herd received a double vaccination (Lysigin™), 30 and 15 days before calving, and 5 months after calving) In conclusion, the current study on commercially available vaccine (Lysigin™), and its potential use as a preventive way for *S. aureus* mastitis in Holstein cows in Egypt showed encouraging results associated with induction of specific and significant antibody responses and significant lower prevalence and incidence of clinical, subclinical and repeated mastitis. The vaccine is considered an additional tool in the control of *S. aureus* infections on farms and that its use should be always associated with excellent farm management practices to successfully improve the infections control within the herd.

Keywords:

Cattle; bacteria; *Staphylococcus aureus; ELISA;* Mastitis; vaccination.

INTRODUCTION

Staphylococcus aureus is one of the most important pathogens, causing clinical and subclinical mastitis in dairy cows and buffaloes all over the world and Egypt; Clinical outcomes and high within-herd prevalence were recently described in dairy cows confirming its relevance as contagious microorganism **(Guccione *et al.,* 2014 and El-Ashker *et al*., 2015)**. Such bacteria typically colonize the injured skin; damage of the teat end and faulty milking encourages migration of bacteria into the udder causing intramammary infections (IMI) and sometimes persisting for extended periods, **Keefe, (2012)**. Some strains are particularly resistant to antibiotics **(Fagiolo and Lai, 2007)** and moderate results were reported **(Guccione *et al.,* 2014)**. Control of *S. aureus* IMI is based on milking-time hygiene; antibiotic therapy and culling of chronically infected cows. The cure rate of *S. aureus* IMI following antibiotic treatment is low and therefore in many herds the disease is not effectively controlled **(Guccione *et al.,* 2014 and Fagiolo and Lai, 2007)**. Due to these limitations, Great scientific attention was recently given to mastitis control by means of preventive vaccination protocols in cows **(Pereira *et al.,* 2011; Daum *et al.,* 2012; Schukken *et al*., 2014; Bradley *et al.,* 2015 and Freick *et al.,* 2015)**. Several experimental immunogens for *S. aureus* mastitis control have been evaluated during the last two decades **(Pereira *et al*., 2011).** Only two vaccines, composed of *S. aureus* strain lysates expressing capsular polysaccharides (CP) adjuvanted with Al $(OH)_3$ (Lysigin™, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) and inactivated *S. aureus* expressing slime-associated antigenic complex formulated with an oil-based adjuvant (Startvac®, Laboratorios Hipra, S.A.), are currently commercially available worldwide. The mechanisms by which whole-cell or lysate vaccines may protect the mammary gland against *S. aureus* have not been fully explored. Since the main goal of *S. aureus* mastitis vaccination is prevention of new intermammary infection **(Middleton, 2008),** immunogens that elicit a long-term antibody response against pathogen factors involved in early host-pathogen interactions should contribute to prevent establishment of the infection. Enhancement of blood polymorph nuclear neutrophil (PMN) phagocytosis through production of antibodies raised against *S. aureus* CP2 bacterial lysates encapsulated in microspheres has been demonstrated **(O'Brien *et al*., 2001).** In addition, it has been shown that *S. aureus* CP5 whole cell and lysate vaccine are able to stimulate strong antibody responses in blood that increase PMN opsonic capacity **(Camussone *et al*., 2014 and Renna *et al*., 2014)**. Day 7 post calving was the time at which the highest specific antibody levels were detected

(Camussone *et al*., 2013, 2014). Macrophages are the major cell type in dry mammary gland secretions, colostrum and milk **(Rainard and Riollet,2006)**; among several other functions, they recognize microorganisms, alert the immune system, recruit PMN and initiate an inflammatory reaction **(Mosser and Edwards,2008).** Macrophage has receptors for IgG_1 and IgG_2 and actively phagocytoses bacterial pathogens **(Desiderio and Campbell, 1980).** The ability of *S. aureus* to attach to and internalize into mammary epithelial cells (MEC) is instrumental to mammary gland colonization and development of intramammary infection **(Camussone *et al*., 2013)**. Several studies have addressed the role of antibodies directed against key antigens involved in adherence to/invasion of mammary epithelial cells **(Nour El-Din *et al*., 2006)**. Sera from pregnant heifers immunized with *S. aureus* CP lysate cells formulated with Al $(OH)_3$ (Lysigin™) stimulated antibodies production that inhibited internalization in (MEC) and increased phagocytosis by milk macrophages, providing insight into the putative mechanism by which this vaccine can affords protection to the mammary gland against *S. aureus* intramammary infection **(Renna *et al.*,2014)**. The use of inactivated polyvalent mastitis vaccine against *Staphylococcus aureus* in buffaloes like in dairy cows showed encouraging results associated with significant lower prevalence and incidence of mastitis as well as lower SCC values **(Guccione *et al.*, 2017)**.The aim of the present study was to evaluate the humoral immunological response to a commercially available vaccine (Lysigin™, Boehringer Ingelheim Vetmedica Inc., and St. Joseph, MO) and its potential use as a preventive way for *S. aureus* mastitis Ferizian cows Vaccinated (V) and not vaccinated (N-V) groups, of 30 cows, were selected from one herd of the same farm. The outcome was evaluated according the obtained results of antibody response by ELISA, and using data on the udder health (clinical, subclinical and repeated mastitis).

MATERIAL AND METHODS

Animals and Farm Management:

A herd of mean 686 (686 ± 20.13) Holstein cows from a dairy farm that is located at Nobaria area - Egypt *and suffered from S. aureus* mastitis problems continuously for at least 3 years, according to the supervising herd veterinarians and owners, was enrolled in the study. Traditional control measures consisting of identification and segregation of infected cows, culling of chronically infected cows, selective dry cow therapy, control of milking equipment and improvement of hygienic measures had been performed but were considered unsuccessful.

The herd had semi opened housing system and milked his cows in a milking parlor twice a day. Information on numbers of cows, milk production, bulk milk SCC, and proportion of cow's veterinary-treated for clinical mastitis (VTCM) the 6 months before the start of the study were recorded in the farm records. Differences observed about herd management practices were recorded during the period of the investigation, from august 2013 to 30 July 2014 to exclude possible influences on vaccination efficacy. The entire herd was fed a total mixed ratio including hay, silage and a multi-vitamin integrator three times a day; free access to the protected water trough was always guaranteed.

Study design:

All heifers and cows were vaccinated three times with 5ml of the vaccine intramuscularly according to the manufacturer's instructions, i.e. 30 days before expected calving, 15 days after the first vaccination and 5 months after the second vaccination. Six pregnant animal groups with total number of sixty heifers and cows, at the last two months of gestation, were used to evaluate the serological specific antibody response of the vaccine and its duration. Ten pregnant heifers (L1), ten pregnant cows in the 2nd lactation season (L2), ten pregnant cows in the 3rd lactation season (L3), ten pregnant cows in the 4th lactation season (L4), ten pregnant cows in the 5th lactation season (L5) and ten pregnant cows remained unvaccinated as control groups (Control) were used for this evaluation. All the animals were individually submitted to a complete clinical examination with particular focus on the udder health status following the clinical procedure described by Ciaramella [20] and with an estimated calving date to allow vaccination at predicted times before calving. All the cows present in the herd 6 months before the start of vaccination were used in the study of the effect of the vaccine on the prevention of mastitis (681 ± 11.69). Information on vaccinated or control groups, expected calving day and day of actual calving was registered for each cow. When occurring, day of treatment clinical mastitis, and day and cause of culling, was also registered by the first author.

Vaccine and vaccination:

Commercial polyvalent mastitis vaccine (Lysigin™, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO). A, lysate culture of highly antigenic polyvalent somatic antigen containing phage types I, II, III, IV and miscellaneous groups of *Staph aureus* adjuvanted with Al $(OH)_3$, includes capsular serotypes 5, 8, 336. The owner was previously informed about the purposes and methods of the study. During the present investigation each cow was submitted to the

same vaccination protocol: based on Three intramuscular vaccine injections located at the medium third of the neck (5 mL each one) and performed at 30 and 15 days before the estimated date of calving and 5 months after calving. Clinical signs were monitored throughout the study by a veterinarian, every 4 h during the first 24 h, and subsequently every time the cows were milked. General attitude and appetite were observed. The udders were palpated for soreness, swelling, hardness and heat, and the appearance of milk was assessed visually for clots and changes in color or composition every time the cows were milked, for 6 months after vaccination. All animals involved in this investigation were cared for in accordance with The International Guiding Principles for Biomedical Research Involving Animals, 1985 **(Pellegrino *et al.*, 2010)**. California Mastitis Test (CMT) was routinely performed from each composite milk sample, with values ≥1 interpreted as positive **(Guccione *et al.*, 2014)** - data not shown.

Blood sampling:

Blood samples were collected from the investigated pregnant heifers and cows immediately before the administration of each dose of vaccine, at calving time and monthly for 11 months after calving. Approximately 30 ml of blood were obtained from the jugular vein into sterile tubes. Samples were maintained at room temperature, centrifuged at 1200×g for 10 min and blood sera collected and stored at -20 ºC until processed for ELISA **(Pellegrino *et al.*, 2010)**.

Detection of antibodies:

The levels of specific antibodies were determined by indirect enzyme-linked immunosorbent assay (ELISA) as previously described **(Tollersrud *et al.*, 2001)**. In brief, 96-well immunoplates (Nunc, Kamstrup, Denmark) were coated with purified CP5 (4 µg/ml) according to **Gray (1979)** and incubated 2 h at 37 ºC and overnight at 4ºC. The coating solution was then removed and the plates were blocked with powdered milk solution (2%) at 37 ºC for 2 h. After washing plates three times with PBS -Tween (PBS 0.1M pH 7.4, 0.5% Tween 20), tested blood sera (1:250) were added in duplicate. The plates were incubated for 2 h at 37 ºC, and then washed three times. For total specific IgG determination, monoclonal alkaline phosphatase labeled mouse anti-bovine IgG (Sigma, Chemical Co., St. Louis, MO) diluted 1:15000 in PBST was used as conjugate and incubated for 2 h at 37 ºC. Substrate was added and the plates were read at OD405 on an ELISA plate reader (Bio-tech XL 808, USA). To each plate, positive and negative serum standards were added. The optical density of each tested sample was recorded. The cut off value was defined as the mean optical density (OD)

value+2 standard errors of the mean (SEM) of all sera at the lowest serum dilution (1:250) at day 0 and OD ≥1was considered protective antibody level.

Statistical analysis:

Results were expressed as means ± standard deviations (SD), and the significance of the differences was measured by the student t-test and ANOVA test for antibody production between control and vaccinated groups. A *p-value* < 0.05 was considered indicative of a statistically significant difference **(Cui *et al.*, 2010)**.

RESULTS

The effect of a systemic immunization against staphylococcal mastitis in Holstein pregnant cows with Commercial polyvalent mastitis vaccine (Lysigin™, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), was investigated. None of the vaccinated cows showed signs of sensitivity to the vaccine except for a small local swelling occurring only up to 2 days after vaccination. The vaccination procedure employed did not affect pregnancy or the health of the new-born calves, with no teratogenic features. No difference could be detected in calf survival rate between the immunized and the control cows. Prior to vaccination, the cows did not have antibodies to *S. aureus*, whereas following vaccination; a significant increase ($p < 0.05$) in specific IgG was detected in the blood sera of all vaccinated cows, reaching a protective level at calving,15-30 days after the revaccination. Antibody to *S. aureus* was not detected in any of the control cows. (Table 1) and Fig. (1). after calving; antibody titer increased and steady, with some fluctuations, but remained at protective level till the administration of the third dose at the 6th month post vaccination. Fig. (1). Heifers and cows in the 2nd lactation season (L2) showed the highest antibody response without significant differences between them, cows in L3 and L4 showed the same antibody response without significant differences between them while cows in the 5th lactation season (L5) showed the lowest antibody response with relative protective level. However, the significant differences were those between revaccinated or vaccinated cows and the controls; heifers or cows in L2, cows in L3 or L4 and cows in L5 and the vaccinated and the revaccinated cows and heifers ($p < 0.05$) Fig. (1). In a comparative study for the effect of the vaccine on mastitis rate among the individuals of the herd during 6 months before and after vaccination, a significant reduction in mastitis percent was observed among cows showing clinical, subclinical or repeated mastitis ($p < 0.05$) Fig. (2). The percent of cows with clinical mastitis, subclinical mastitis and repeated mastitis was reduced from 9.98 ± 0.52, 12.19 ± 0.63 and 16.56 ± 1.17 to

5.40 ± 0.28, 3.51 ± 0.41 and 1.64 ± 0.88 respectively, the total mastitis rate percent reduced from 22.17 ± 0.57 to 8.91 ± 0.69 (Table 2).

DISCUSSION

Mastitis caused by *S. aureus* remains the most costly disease for dairy farmers. Although antibiotic therapy and culling of infected animals are the most approaches to control of the disease, the success rate of treatment against *S. aureus* infections varies considerably **(Perez-Casal *et al.,* 2006)**. It is well established that immune-suppression of cows during the periparturient period considerably increases the incidence of mastitis in early lactation and many attempts have been made to improve the resistance of animals to (IMI) during this period **(Sordillo and Streicher,2002; Sordillo, 2005;McDougall *et al.,* 2009 and Middleton *et al.,* 2009)**. Therefore, the application of immune modulatory strategies for the control of *S. aureus* mastitis outbreaks in dairy herds during this period is critical, and is an alternative to treatment with antibiotics, which may lead to the entry of antibiotic resistance bacteria into the food chain **(Leitner *et al*., 2003b ; Perez-Casal *et al.,* 2006 and Pellegrino *et al.,* 2010)**. Many authors demonstrated that, the antibodies generated by a *S. aureus* CP lysate vaccines adjuvanted with $Al(OH)_3$, significantly reduced bacterial adherence and internalization to primary mammary secretory epithelial cells and could block early host-pathogen interactions and favor macrophages and neutrophil phagocytic activity, thus helping to clear the organism from the mammary gland and prevent intramamary infection **(Risley *et al*., 2007; Middleton *et al*., 2009; Camussone *et al*., 2013 and Renna *et al*., 2014)**. In this study we evaluated the humoral response to a commercially available *Staphylococcus aureus* vaccine (Lysigin) generated in pregnant heifers and cows, in several lactation seasons, during immunization. Estimation of vaccine effects against mastitis under field conditions was one of our goals to aid in the control of *S. aureus* mastitis in dairy cows in Egypt. Vaccination with *Staphylococcus aureus* vaccine (Lysigin) in this study, did not affect pregnancy and no difference was observed between vaccinated and control heifers and cows in the numbers of healthy calves delivered. Our findings are in agreement with the data published **(Leitner *et al.,* 2003)**. In contrast, vaccination with a commercial polyvalent vaccine did not have any beneficial effects on udder health or survival in two commercial dairy herds with mastitis problems due to *S. aureus* **(Landin *et al.,* 2015)**. All heifers and cows under investigation, responded to the (Lysigin) with production of protective specific antibodies. Heifers and cows in the 2nd lactation season (L2) showed the highest antibody response without significant

differences between them. It is well known that the magnitude, duration and effectiveness of mammary gland immunity depend of the antigens used in vaccination **(Calzolari *et al.,* 1997; Nour El-Din *et al.,* 2006 and Chang *et al.,* 2008)**. The high levels of specific antibodies in blood of vaccinated heifers and cows peripartum Fig. (1) Suggests that, the vaccination protocol used and the particular properties of the CP lysate allowed a strong immunogenic effect for a long period of time (5-6months). The highest level (2-fold) found in specific IgG in blood of vaccinated heifers at calving was achieved after 2 intramuscular doses of the vaccine. It is known that specific antibodies present in milk are mostly transported from blood to the udder after vaccination **(Sordillo, 2005)**. The significant increase in the levels of specific IgG antibodies in blood between days 15 and 30 (Table 1) after calving could be due to activation and proliferation of blood memory cells previously stimulated. The results are in line with **(Leitner *et al.,* 2003; Pereira *et al.,* 2011 and Schukken *et al.,*2014)** who found a reduced duration of *S. aureus* intramammary infection in vaccinated cows, and that vaccine efficacy was better in primiparous cows than in older cows. IgG antibodies play an important role as opsonin for phagocytosis, during mammary gland infection **(Prenafeta *et al*., 2010).** Several studies have evaluated the opsonic capacity of antibodies raised against *S. aureus* whole-cell or CP lysate vaccines for bovine blood poly morph nucleic cells PMN **(Camussone *et al*., 2013, 2014).** An increase in the percentage of milk macrophages that phagocytosed *S. aureus*, and in the number of bacteria ingested per cell, was observed in cows vaccinated with *S. aureus* CP lysate or *S. aureus* bacterin, this is attributed to the increase in opsonization due to high anti-CP IgG levels in the vaccinated cows **(Camussone *et al*., 2014)**. A, vaccination booster with whole cells and lysates shown to achieve high antibody levels at the end of lactation **(Camussone *et al*., 2014)** could therefore favor macrophage activity during this period and protect against mastitis. Our estimation of vaccine effects against mastitis under field conditions revealed a significant reduction in mastitis percent among cows showing clinical, subclinical or repeated mastitis (Table 2) and Fig.(2).Our observations are in agreement with However, **(Bradley *et al.,* 2015)** who found that vaccinated cows were less likely to have severe clinical mastitis than unvaccinated cows, and **(Guccione *et al.,* 2017)** who reported a significant difference in the overall rate of mastitis, comparing unvaccinated and vaccinated heifers and cows, and administrations of 3 doses of vaccine, may significantly reduce the incidence of mastitis within the herd. Therefore, the novel contribution of the current study has been to show encouraging results regarding the efficacy of the used

commercially available *Staphylococcus aureus* vaccine (Lysigin) on heifers and cows in Egypt in contrast with some previous similar studies on staphylococcal vaccination in dairy cows described relatively low vaccine efficacy or no vaccine efficacy at all **(Middleton *et al.*, 2009; Hardarson and Sveinbj◌rnsson, 2013; Kalmus *et al.*, 2013 and Landin *et al.*, 2015)**. In conclusion, the current study represents the first investigation evaluating direct effects of vaccination against *S. aureus* in dairy cows in Egypt. commercially available *Staphylococcus* aureus vaccine,Lysigin™,(based on label use) showsed encouraging results because associated with induction of specific and significant antibody responses and significant lower prevalence and incidence of clinical, subclinical and repeated mastitis. The vaccine is considered an additional tool in the control of *S. aureus* infections on farms and that its use should be always associated with excellent farm management practices to successfully improve the infections control within the herd.

REFERENCES

Bradley AJ, Breen JE, Payne B, White V, Green and MJ. (2015): An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom. J Dairy Sci. 2015; 98: 1706 - 20.

Calzolari A, Giraudo JE, Rampone H, Odierno L, Giraudo AT and Frigerio C. (1997): Field trials in Argentina of a vaccine against bovine mastitis. 2. Evaluation in two dairy herds. J Dairy Sci 1997; 80:854 - 8.

Camussone CM, Veaute CM, Porporatto C, Morein B, Marcipar IS and Calvinho LF. (2013): Immune response of heifers against a *Staphylococcus aureus* CP5 whole cell vaccine formulated with ISCOM MATRIXTM adjuvant. J Dairy Res 80: 72-80.

Camussone CM, Veaute CM, Pujato N, Morein B, Marcipar IS and Calvinho LF. (2014): Immune response of heifers against a *Staphylococcus aureus* CP5 whole cell and lysate vaccine formulated with Iscom Matrix adjuvant. Res Vet Sci 96: 86-94.

Chang BS, San Moon J, Kang H, Kim Y, Lee H and Kim J. (2008): Protective effects of recombinant staphylococcal enterotoxin type C mutant vaccine against experimental bovine infection by a strain of *Staphylococcus aureus* isolated from subclinical mastitis in dairy cattle. Vaccine 2008; 26:2081-91.

Cui JC, Zhang BJ, Lin YC, Wang QK, Qian AD, Nakane A, Hu DL and Tong GZ. (2010): Protective effect of glutathione S-transferase fused mutant staphylococcal enterotoxin C against *Staphylococcus aureus*-induced bovine mastitis. Veterinary Immunology and Immunopathology 135 (2010) 64 -70.

Daum RS and Spellberg B. (2012): Progress toward a *Staphylococcus aureus* vaccine. Clin Infect Dis. 2012; 54:560 - 7.

Desiderio JVand Campbell SG.(1980): Bovine mammary gland macrophage: isolation, morphologic features, and cytophilic immunoglobulins. Am J Vet Res 41:1595-1599.

El-Ashker M, Gwida M, Tomaso H, Monecke S, Ehricht R, El-Gohary F, Hotzel H.(2015): Staphylococci in cattle and buffaloes with mastitis in Dakahlia Governorate, Egypt. J Dairy Sci. 2015 Nov; 98 (11):7450-9.

Fagiolo A and Lai O. (2007): Mastitis in buffalo. Ital J Anim Sci. 2007; 6:200 - 6.

Freick M,Frank Y,Steinert K, Hamedy A, Passarge O and Sobiraj A. (2016): Mastitis vaccination using a commercial polyvalent vaccine or a herd specific *Staphylococcus aureus* vaccine. Results of a controlled field trial on a dairy farm. Tierarztl Prax Ausg G Grosstiere Nutztiere. 2016; 44:219 -29.

Gray BM. ELISA methodology for polysaccharide antigens (1979): protein coupling of polysaccharides for adsorption to plastic tubes. J Immunol Methods 1979; 28:187-92.

Guccione J, Cosandey A, Pesce A, Di Loria A, Pascale M and Piantedosi D. (2014): Clinical outcomes and molecular genotyping of *Staphylococcus aureus* isolated from milk samples of dairy primiparous Mediterranean buffaloes (Bubalus bubalis). J Dairy Sci. 2014; 97:7606 -13.

Guccione J, Pesce A, Pascale M, Salzano C, Tedeschi G, D'Andrea L, De Rosa A and Ciaramella P. (2017): Efficacy of a polyvalent mastitis vaccine against *Staphylococcus aureus* on a dairy Mediterranean buffalo farm: results of two clinical field trials. BMC Veterinary Research (2017) 13:29.

Guccione J, Pesce A, Pascale M, Tommasini N, Garofalo F and Loria A. (2014): Short communication: Effects of systemic treatment with penethamate hydriodide on udder health and milk yields in dry primiparous Mediterranean buffaloes (Bubalus bubalis). J Dairy Sci. 2014; 97:2219 -25.

Hardarson HG and Sveinbj̨rnsson J. (2013): The effect of Startvac® on udder health under Icelandic conditions. In: Proceedings of the NKV ET Symposium 13-14 May 2013; Reykjavik; 2013:28.

Kalmus P, Simojoki H and Py̨örälä S. (2013): Vaccination with a commercial mastitis vaccine Startvac® did not affect the incidence of clinical mastitis in a large dairy herd. In: Proceedings of the NKVet Symposium 13-14 May 2013; Reykjavik; 2013:37.

Keefe G. (2012): Update on control of *Staphylococcus aureus* and *Streptococcus agalactiae* for management of mastitis. Vet Clin North Am Food Anim Pract. 2012; 28:203 - 16.

Landin H, M̨örk MJ, Larsson M and Waller KP. (2015): Vaccination against *Staphylococcus aureus* mastitis in two Swedish dairy herds. *Acta Vet Scand* (2015) *57:81.*

Leitner G, Yadlin N , Lubashevsy E, Ezra E, Glickman A, Chaffer M, Winkler M, Saran A and Trainin Z. (2003): Development of a *Staphylococcus aureus* vaccine against mastitis in dairy cows. II. Field trial. Veterinary Immunology and Immunopathology 93 (2003) 153-158.

Leitner, G., Yadlin, N., Lubashevsky, E., Ezra, E., Glickman, A., Chaffer, M., Winkler, M., Saran, A. and Trainin, Z. (2003b): Development of a *Staphylococcus aureus* vaccine against mastitis in dairy cows. II. Field trial. Vet. Immunol. Immunopathol. 93, 153-158.

McDougall S, Parker KI, Heuer C and Compton CWR. (2009): Review of prevention and control of heifer mastitis via non-antibiotic strategies. Vet Microbiol 2009; 154:177 - 85.

Middleton JR. (2008): *Staphylococcus aureus* antigens and challenges in vaccine development. Expert Rev Vaccines 7: 805 -815.

Middleton JR, Luby CD and Adams DS. (2009): Efficacy of vaccination against staphylococcal mastitis: a review and new data. Vet Microbiol 2009; 134:192 - 8.

Mosser DM and Edwards JP. (2008): Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8: 958 - 969.

Nour El-Din AN, Shkreta L, Talbot BG, Diarra MS and Lacasse P. (2006): DNA immunization of dairy cows with the clumping factor A of *Staphylococcus aureus*. Vaccine 24:1997-2006.

O'Brien CN,GuidryAJ,Douglass LW and Westhoff DC.(2001): Immunization with *Staphylococcus aureus* lysate incorporated into microspheres. J Dairy Sci 84: 1791-1799.

Pellegrino M, Giraudo J, Raspanti C, Odierno L and Bogni C. (2010): Efficacy of immunization against bovine mastitis using a *Staphylococcus aureus* avirulent mutant vaccine. Vaccine 28 (2010) 4523 - 4528.

Pereira UP, Oliveira DGS, Mesquita LR, Costa GM and Pereira LJ. (2011): Efficacy of *Staphylococcus aureus* vaccines for bovine mastitis: a systematic review. Vet Microbiol 148: 117-124.

Perez-Casal J, Prysliak T, Kerro-Dego O and Potter AA. (2006): Immune responses to a *Staphylococcus aureus* GapC/B chimera and its potential use as a component of vaccine for S. aureus mastitis. Veterinary Immunology and Immunopathology 109 (2006) 85-97.

Prenafeta A, March R, Foix A, Casals I and Costa L. (2010): Study of the humoral immunological response after vaccination with a *Staphylococcus aureus* biofilm embedded bacterin in dairy cows: possible role of the exopolysaccharide specific antibody production in the protection from *Staphylococcus* aureus induced mastitis. Vet Immunol Immunopathol 134: 208 - 217.

Rainard P and Riollet C. (2006): Innate immunity of the bovine mammary gland. Vet Res 37: 369 - 400.

Renna MS, Pereyra EAl, Baravalle C, and Cecilia M. Camussone CM, Dallard BE, Marcipar IS and Calvinho LF. (2014): Functional role of antibodies generated in heifers through immunization with *Staphylococcus aureus* vaccines in invasion and phagocytosis assays. FEMS Microbiol Lett 360 (2014) 62 - 69.

Risley AL, Loughman A, Cywes-Bentley C, Foster TJ and Lee JC. (2007): Capsular polysaccharide masks clumping factor A-mediated adherence of *Staphylococcus aureus* to fibrinogen and platelets. J Infect Dis 196: 919 - 927.

Schukken YH, Bronzo V, Locatelli C, Pollera C, Rota N and Casula A.(2014): Efficacy of vaccination on *Staphylococcus aureus* and coagulase-negative staphylococci intramammary infection dynamics in 2 dairy herds. J Dairy Sci. 2014; 97:5250 - 64.

Sordillo LM and Streicher KL. (2002): Mammary gland immunity and mastitis susceptibility. J Mammary Gland Biol Neoplasia 2002; 7 (2):135 - 46.

Sordillo LM. (2005): Factors affecting mammary gland immunity and mastitis susceptibility. Livest Prod Sci 2005; 98:89 - 99.

Tollersrud T, Zernichow L, Andersen SR, Kenny K and Lund A. (2001): *Staphylococcus aureus* capsular polysaccharide type 5 conjugate and whole cell vaccines stimulate antibody responses in cattle. Vaccine 19 (2001) 3896 -3903.

Table (No.1): The Mean Optical density (OD) of Serum Antibodies from pregnant heifers and cows in different lactation seasons vaccinated with "Lysigin" vaccine using ELISA.

Lactating Group No.	Before calving		After calving											
	1st Dose	2nd Dose	3rd Dose											
	1-Aug-2013	15-Aug-2013	30-Aug-2013	30-Sep-2013	30-Oct-2013	30-Nov-2013	30-Dec-2013	30-Jan-2014	28-Feb-2014	30-Mar-2014	30-Apr-2014	30-May-2014	30-Jun-2014	30-Jul-2014
L.1 Group	0.32 (0.02)	0.84 (0.02)	1.11 (0.02)	1.22 (0.05)	1.71 (0.12)	1.69 (0.11)	1.73 (0.12)	1.69 (0.09)	1.31 (0.05)	1.68 (0.05)	1.88 (0.04)	1.85 (0.05)	1.86 (0.04)	1.69 (0.03)
L.2 Group	0.34 (0.01)	0.79 (0.01)	1.09. (0.06)	1.13 (0.06)	1.68 (0.03)	1.62 (0.03)	1.70 (0.03)	1.61 (0.02)	1.22 (0.03)	1.61 (0.03)	1.79 (0.02)	1.79 (0.02)	1.77 (0.03)	1.58 (0.02)
L.3 Group	0.33 (0.02)	0.60 (0.02)	0.78 (0.03)	1.00 (0.03)	1.52 (0.02)	1.48 (0.03)	1.61 (0.03)	1.39 (0.03)	1.00 (0.06)	1.41 (0.02)	1.62 (0.03)	1.61 (0.03)	1.61 (0.03)	1.38 (0.02)
L.4 Group	0.31 (0.01)	0.58 (0.05)	0.75 (0.05)	1.00 (0.05)	1.48 (0.05)	1.44 (0.03)	1.49 (0.02)	1.34 (0.03)	1.00 (0.05)	1.38 (0.02)	1.56 (0.02)	1.56 (0.0)	1.50 (0.03)	1.31 (0.05)
L.5 Group	0.31 (0.01)	0.47 (0.03)	0.64 (0.06)	0.98 (0.05)	1.21 (0.04)	1.31 (0.10)	1.30 (0.03)	1.28 (0.03)	0.99 (0.065)	1.13 (0.01)	1.11 (0.01)	1.11 (0.01)	1.01 (0.02)	0.98 (0.02)
Control Group	0.32 (0.01)	0.32 (0.01)	0.32 (0.01)	0.31 (0.01)	0.31 (0.01)	0.32 (0.01)	0.32 (0.01)	0.32 (0.01)	0.32 (0.01)	0.32 (0.01)	0.32 (0.01)	0.32 (0.01)	0.32 (0.01)	0.32 (0.01)

L. = lactation season

Table (No. 2): Effect of vaccination on mastitis rate.

	Before vaccination (Before Calving)		After vaccination (After Calving)	
	Numbers of Cows	Cow Percent (%)	Numbers of Cows	Cow Percent (%)
Mastetic cows %	68 ± 4.08	9.98 ± 0.52	37 ± 1.41	5.40 ± 0.28
Subclinical cows %	83 ± 4.12	12.19 ± 0.63	24 ± 2.31	3.51 ± 0.41
Total mastitic cows %	151 ± 4.66	22.17 ± 0.57	61 ± 3.56	8.91 ± 0.69
Repeated cows %	25 ±1.92 /151	16.56 ± 1.17	1 ± 0.58 / 61	1.64 ± 0.88

Mean herd size before vaccination = 681 ± 11.69

Mean herd size after vaccination = 686 ± 20.13

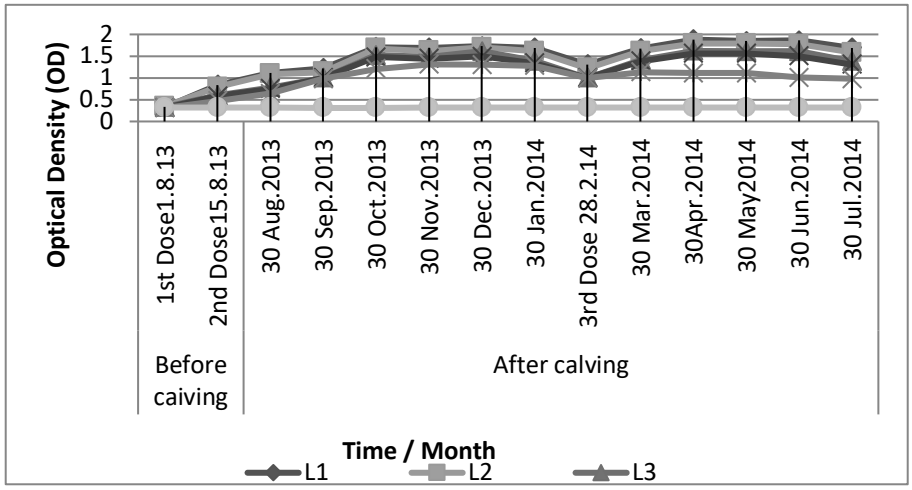

Fig. (No.1): Optical density of serum antibody response of pregnant heifers and cows in different lactation seasons vaccinated with "Lysigin" vaccine using ELISA.

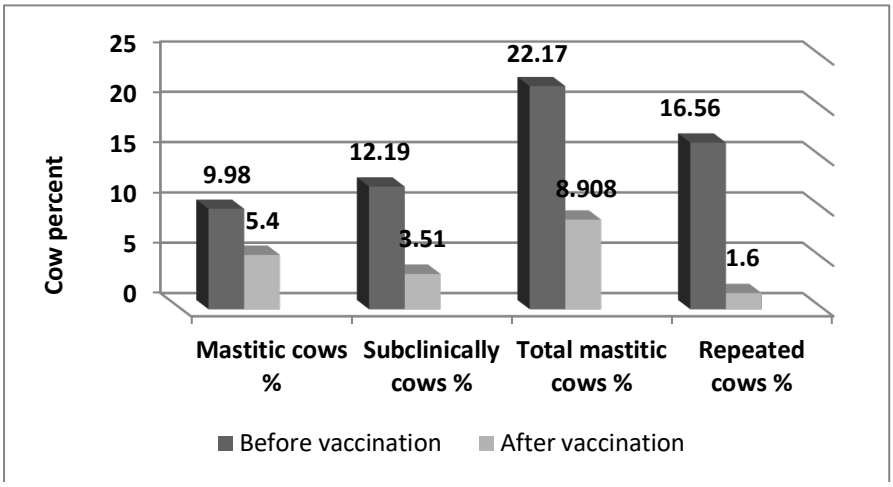

Fig. (No.2): Clinical, subclinical and repeated mastitis before and after vaccination.