Efficacy of Lysigin Vaccine in the Prevention of Mastitis in Dairy Cattle

Ahmed A. Badr[1], Noha M. Bakry[2*], Khaled F. Al-Amry[3] and Samia A. Ahmed[2]

1. Post graduate student, Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
2. Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
3. Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

* Corresponding author: Noha M. Bakry, Email: noha_nady90@cu.edu.eg

1. Abstract

Mastitis is one of the biggest expenses for the dairy industry which has a substantial impact on dairy cow health. Preventing new infections in cows is the best strategy to manage mastitis. This study aimed to investigate the effect of a commercially available *S. aureus* bacterin (Lysigin) on minimizing the rate of mastitis in a dairy herd. A total number of 600 Holstein-Friesian dairy cows were involved in the study and received two doses of the vaccine. The enrolled animals were monitored for clinical signs, bacterial culture, somatic cell count (SCC), and costs utilized for treatment during the study. The rate of clinical and subclinical mastitis was reduced from 13% and 29% to 6.7% and 18.3%, respectively after one year following vaccination. Composite milk samples from infected cows were examined for bacteriological isolation of *S. aureus* and *E. coli*. *S. aureus* and *E. coli* mastitis were determined in percentages of 25% and 30.9%, respectively. Following immunization, *S. aureus* and *E. coli* mastitis were decreased to 10% and 23.3%, respectively. The decrease in the rate of *E. coli* may be related to farm hygiene and environmental management which has a detrimental effect in the control of coliform mastitis. The SCC on milk samples from animals with chronic infection significantly decreased after vaccination. Furthermore, a reduction in bulk milk tank SSC (BTSSC) was detected in the herd during the study. The application of vaccination has a greater impact on reducing the costs utilized for the treatment of mastitis in the herd. These findings indicate that the Lysigin vaccine has a protective effect against *S. aureus* mastitis and can be utilized as an additional approach for the management of mastitis.

Keywords: Clinical mastitis, Dairy cattle, Lysigin vaccine, *S. aureus*, Somatic cell count, Subclinical mastitis

2. Introduction

Bovine mastitis is a frequently occurring and economically significant disease that affects dairy cattle production worldwide. It is the most costly disease in the dairy sector, contributing to significant financial losses as a consequence of reduced milk production, alteration in milk composition, discarded milk, and culling of chronically infected cows, in addition to more substantial veterinary, diagnostic, and treatment expenses [1,2].

The most prominent forms of mastitis are clinical mastitis which is typically identified through particular physical changes in the milk and pathological changes on the udder, and sub-clinical mastitis, in which no observable adulteration in the milk is visible [3, 4]. The prevalence of subclinical mastitis (SCM) is anticipated to be 15 to 40 times more frequent than clinical instances [5]. Both forms of mastitis reduce milk production, however subclinical mastitis is relatively more prevalent and entails more substantial financial losses than its clinical counterpart [6, 7].

The primary cause of bovine mastitis is bacterial intramammary infection (IMI), which can be categorized into either contagious or environmental pathogens [8]. The vast majority of mastitis occurrence is mainly attributed to the infection with different species of *Staphylococcus* species, *Streptococcus* species, and coliform bacteria [9]. *Staphylococcus aureus* is a well-known contagious pathogen that is responsible for subclinical or chronic mastitis in dairy cows, resulting in enormous economic losses [10]. Furthermore, coliform bacteria are a frequent cause of bovine clinical mastitis, particularly *E. coli* which accounts for more than 80% of coliform mastitis instances [11, 12].

The somatic cell count (SCC) is an essential measure of milk quality and an indicator employed for monitoring mastitis, especially in its subclinical form [13]. Cows with a count less than 200,000 cells/ml can be considered healthy or to have recovered from mastitis, however, intramammary infections are more probable to be encountered when the SCC rate exceeds 400,000 cells/ml [14].

The cure rate of antimicrobial therapy for mastitis pathogens, particularly *S. aureus* is very poor, and the extensive use of antibiotics for the treatment of mastitis has culminated in the emergence of highly resistant bacterial strains causing mastitis control to be more challenging [15]. During the last years, vaccination against *S. aureus* mastitis has been investigated and advocated as an essential approach to combat staphylococcal infections in dairy cows [`16, 17, 18]. Lysigin and Somato-Staph are commercially available *S. aureus* bacterins in the United States to control bovine mastitis against *S. aureus* [19]. Moreover, a vaccine against *S. aureus* and *E. coli* (Startvac), which also targets coagulase-negative staphylococci, has been developed [17].

Regardless of the vaccine type, vaccination alone is inadequate for preventing mastitis, particularly in dairy herds with high mastitis rates [20]. Therefore, vaccination as a good control strategy must be complemented with other traditional control programs focusing on hygiene and management to reduce the frequency and duration of mastitis cases [17, 21]. The purpose of this study was to investigate the effect of vaccination with a commercially available *S. aureus* bacterin (Lysigin, Boehringer Ingelheim Vetmedica, Inc.) to reduce the rate of mastitis infection in a dairy farm in Egypt.

3. Materials and Methods

3.1. Ethical approval

The study was approved and carried out in accordance with the ethics operational guidelines of the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Cairo University with an approval number (VET CU

08072023676).

3.2. Animals

This study was conducted on a private dairy farm located in El-Gharbia governorate during the period from 2014 till 2016. A herd of 600 Holstein-Friesian dairy cows were examined and enrolled in the study. The mammary glands and teats were manually palpated for detection of any abnormalities. Alterations of milk secretion including the presence of clots, blood, or pus were recorded during the investigation [22].

3.3. Vaccination

A commercially available *S. aureus* mastitis bacterin (Lysigin®, Boehringer Ingelheim Vetmedica, Inc.) was utilized and evaluated to control bovine mastitis against *S. aureus* under field condition. This bacterin contains a lysate culture of highly antigenic polyvalent somatic antigen including 5 phage types and 5, 8, 336 capsular serotype of *S. aureus*. The vaccine was applied in two doses within 3 weeks intervals at any stage of lactation according to the labeled guidelines. Each cow in the dairy farm was injected with 5 ml of lysigin dose intramuscularly. The enrolled 600 cows then received a booster dose of the Lysigin vaccine annually for two years during the study. Composite milk samples were collected for bacterial isolation and measurement of SCC before and after immunization.

3.4. Milk sampling

A total number of 600 composite milk samples from each cow were aseptically collected before and after vaccination according to the International Dairy Federation recommendation [23]. In brief, the teat ends were thoroughly washed and sanitized, the first streams of foremilk were then discarded, and about 10 ml of milk was collected aseptically into sterile vials. All the obtained milk samples were stored at 4°C until bacteriological isolation.

3.5. California Mastitis Test (CMT)

The California mastitis test (CMT) was employed to screen dairy cows for subclinical mastitis (SCM) before and after Lysigin vaccination, following the protocols outlined by Schalm et al. [24]. According to the Adkins and Middleton [25], the results of CMT were assessed as negative, trace, 1+, 2+, or 3+ .

3.6. Bacterial culture

A microbiological culture was performed on composite milk samples which derived from 252 infected cows before vaccination and 150 infected cows after one year post vaccination following the standard milk sample testing protocols stipulated by the National Mastitis Council [26]. The milk samples were incubated for 18-24 hrs at 37 °C then a loopful of the incubated milk was cultured onto Blood agar media containing 5% sheep blood, Mannitol salt agar, and MacConkey agar (Oxoid, UK). All plates were incubated at 37 °C for 24-48 hrs and examined for bacterial growth. Further microscopical and biochemical identification were conducted on suspected colonies of S. *aureus* and *E. coli* [27].

Online ISSN: 2537-1045
Print ISSN: 1110-1423

3.7. Somatic cell count (SCC)

A total number of 35 composite milk samples from dairy cows with chronic infection and bulk milk tank were collected annually during a 3 years period of the study and submitted to the Department of Mastitis and Neonatal Diseases, Animal Reproduction Research Institute (ARRI), Giza, Egypt for detection of SCC. The milk samples were examined for somatic cell count automatically using SomaCount ™ FC (Bentley, USA) according to the International Dairy Federation [28].

3.8. Determination of treatment costs for mastitis

The cost of therapy, including the use of antibiotics, non-steroidal anti-inflammatory drugs (NSAID), and intramammary infusions, was documented for one year before vaccination and two years following vaccination to evaluate the efficacy of the vaccine on the reduction of mastitis economic losses in the investigated animals.

4. Results

The influence of vaccinating Holstein dairy cows against staphylococcal mastitis with a commercial polyvalent mastitis vaccine (Lysigin®) was investigated. This study was conducted on composite milk samples obtained from 600 dairy cows in a private dairy farm. The enrolled animals were vaccinated annually for three years during the study. Clinical examination, CMT application, SCC, and microbiological culture were carried out on the enrolled animals before and after vaccination.

The clinical examination of dairy cows suffering from clinical mastitis shows visible signs such as the udder being red, hot, painful, and swollen, as well as their milk being bloody, watery, or containing flakes, clotted secretion, or pus. In certain cases, elevated body temperature ranging from 39.5 to 40ºC was detected, causing the animal to become off food and depressed. On the contrary, cows with subclinical mastitis did not exhibit any clinical signs either general or localized signs on the udder. A california mastitis test (CMT) was applied to ascertain the rate of subclinical mastitis. According to clinical examination and CMT, the animals before vaccination were grouped into clinically diseased, subclinically infected, and healthy cows in percentages of 13%, 29%, and 58%, respectively (Table 1). Following vaccination, there was a decrease in the rate of clinical and subclinical mastitis, which were estimated at 6.7% and 18.3%, respectively.

Bacteriological examination of composite milk samples from clinically mastitic and subclinically infected animals on a dairy farm was performed before and after vaccination (Table 2). A total number of 252 composite milk samples were examined bacteriologically for isolation and identification of *S. aureus* and *E. coli*. The results revealed the presence of *S. aureus* and *E. coli* in percentages of 25% and 30.9%, respectively. On the contrary, the bacterial culture of composite milk samples from 150 infected cows one year following vaccination demonstrated a marked drop in the rate of infection with *S. aureus* to 10%, while the rate of *E. coli* decreased to 23.3%.

Online ISSN: 2537-1045
Print ISSN: 1110-1423

Measurement of SSC as a diagnostic test for subclinical mastitis was applied on composite milk samples from 35 cows that suffered from chronic mastitis on the farm. The results revealed a marked decline in SCC in chronically infected cows for two years after vaccination with Lysigin as shown in table (3).

Furthermore, SSC analysis on bulk milk tank was employed every month for three years and demonstrated a decrease in BMTSCC two years after vaccination as shown in table (4). The average of BMTSSC before vaccine application was 546,330 ml/cell, however, in the first- and second-year following vaccination, the average count decreased to 234,500 and 210,000 ml/cell, respectively.

In the current study, the costs of field-used antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), and intramammary infusions were estimated before and after vaccination. The most frequently prescribed antibiotics for systemic and intramammary application were amoxicillin-clavulanic acid, marbofloxacin, and cefquinome which were related to the aminopenicillin-beta-lactamase inhibitor, fluoroquinolone, and fourth generation cephalosporins antibiotic classes, respectively. Furthermore, meloxicam, flunixin meglumine, and tolfenamic acid were the most common NSAIDs employed on the farm. As demonstrated in table (5), the application of the Lysigin vaccine has a favorable effect on protecting the animal against mastitis and thereby reducing the costs associated with the herd's mastitis treatment.

5. Discussion

Reducing the occurrence of mastitis is one of the most crucial objectives of dairy farms. *Staphylococcus aureus* is a major pathogen responsible for both clinical and subclinical mastitis in dairy cows [29]. The inability of the current antibiotics to manage the contagious *S. aureus* mastitis, as well as the growing worry about the continual development of antimicrobial resistance in milk highlight the need for antibiotic alternatives [21]. Vaccination against *S. aureus* mastitis has been investigated and advocated as a substantial helpful strategy for the management of staphylococcal infections in dairy cows [16]. The purpose of this study was to investigate the efficacy of administering a commercially available *S. aureus* mastitis bacterin (Lysigin, Boehringer Ingelheim Vetmedica, Inc.) for three years on decreasing the rate of mastitis in dairy cows in Egypt.

In the present study, dairy cows at any stage of lactation received two doses of the Lysigin vaccine at 2 weeks intervals. Collection of milk samples for bacterial isolation and measurement of SCC before and after vaccination were applied. The field-based evaluation of vaccine protection against mastitis demonstrated a reduction in the rate of clinical and subclinical mastitis within the animal herd after vaccination. These findings are consistent with a previous study that demonstrated the effect of the Lysigin vaccine on reducing the progression of clinical symptoms and diminishing the occurrence of subclinical mastitis [30]. Another study concluded that a group of cows vaccinated with Lysigin had a shorter duration of clinical mastitis and a lower total mastitis score than the control group [31]. Furthermore,

Online ISSN: 2537-1045
Print ISSN: 1110-1423

Eisa et al. [32] revealed that in the Lysigin-vaccinated group, the rates of clinical, subclinical, and recurrent mastitis decreased from 9.98%, 12.19%, and 16.56% to 5.4%, 3.5%, and 1.6%, respectively.

Contrary to our results, Tenhagen et al. [33] perceived that this commercial vaccine has a limited potential to prevent new infections and has no meaningful influence on the rate of clinical mastitis, despite eliciting a strong short-term immune response. In addition, immunization with this vaccine in two dairy herds affected with *S. aureus* mastitis did not have any detrimental benefits on udder health, according to a prior investigation [34].

Regarding bacteriological examination, *S. aureus* infection was identified at a lower rate following vaccination. According to a prior study, Lysigin may be useful in minimizing staphylococcal mastitis in periparturient heifers. They reported that vaccinated heifers had a 45% reduction in *S. aureus* intramammary infection compared to the control group [35]. Furthermore, Ghobrial et al. [36] investigated that Lysigin was successfully effective in eliminating 20% of *S. aureus* mastitis in an Egyptian dairy farm. On the other hand, previous results indicate that the vaccine does not entirely protect the udder against *S. aureus* mastitis [21].

Several studies have demonstrated that the application of different commercial *S. aureus* vaccines reduces *S. aureus* intramammary infection in dairy herds [16, 17, 37]. The high concentration of specific antibodies generated against the vaccine strains certainly contributed to a reduction in the frequency of *S. aureus* mastitis [38]. This can be attributed to the vaccine's effect on stimulating the synthesis of anti-*S. aureus* immunoglobulin G2, which is the primary immunoglobulin of the mammary gland immune system and boosts phagocytic activity, resulting in the digestion of engulfed bacteria [39, 40].

In the present study, there was a decrease in the rate of *E. coli* infection following vaccination, from 30.9% to 23.3%. This could be attributable to more frequent cleaning of milking equipment, enhanced milking hygiene practices, and the implementation of disinfectant teat dipping which reduce the rate of *E. coli* infection in this farm. Farm hygiene and environmental management are the cornerstones of coliform mastitis control [41].

Furthermore, this vaccine proved efficacy in minimizing SCC in chronic cases as well as bulk tank milk samples. This was in line with Nickerson et al. [35], who recorded that Lysigin vaccination reduces the severity of mastitis and the somatic cell count in the milk. On the contrary, several studies reported no significant differences in SCC or milk production between vaccinated and non-vaccinated dairy cows [19, 30, 31].

6. Conclusion

According to the findings of this study, it can be concluded that the commercially available *S. aureus* bacterin (Lysigin) had the ability to reduce the rate of clinical and subclinical mastitis in vaccinated dairy cows and had a protective impact against *S. aureus* intramammary infection. The vaccine is regarded as an additional preventive

Online ISSN: 2537-1045
Print ISSN: 1110-1423

strategy in the control of *S. aureus* infections on farms. The effective control of mastitis in dairy herds necessitates the combination of vaccination and appropriate farm management approaches.

Conflict of interest
The authors declare that they have no conflicts of interest

7. **References**

1. Hogeveen H, Huijps K, Lam TJ. Economic aspects of mastitis: new developments. N Z Vet J. 2011;59(1):16-23.

2. Rainard P, Gilbert FB, Germon P, Foucras G. Invited review: a critical appraisal of mastitis vaccines for dairy cows. J Dairy Sci. 2021;104(10):10427-48.

3. Gianneechini R, Concha C, Rivero R, Delucci I, López JM. Occurrence of clinical and sub-clinical mastitis in dairy herds in the West Littoral Region in Uruguay. Acta Vet. Scand. 2002;43(4):1

4. Bhumarkar R, Mahajan G, Kumar A. Doubling farmers income and attaining resilience in agriculture through crop diversification. Agriculture & Food: E-Newsletter. 2021.

5. Seegers H, Fourichon C, Beaudeau F. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet Res. 2003;34(5):475-91.

6. Mdegela RH, Ryoba R, Karimuribo ED, Phiri EJ, Loken T, Reksen O, et al. Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania. J S Afr Vet Assoc. 2009 Sep 1;80(3):163-8.

7. Motaung TE, Petrovski KR, Petzer IM, Thekisoe O, Tsilo TJ. Importance of bovine mastitis in Africa. Anim Health Res Rev. 2017;18(1):58-69.

8. Lakew BT, Fayera T, Ali YM. Risk factors for bovine mastitis with the isolation and identification of *Streptococcus agalactiae* from farms in and around Haramaya district, eastern Ethiopia. Trop Anim Health Prod. 2019;51:1507-13.

9. Blowey RW, Edmondson P. Mastitis control in dairy herds. Cabi; 2010.

10. Godden SM, Jansen JT, Leslie KE, Smart NL, Kelton DF. The effect of sampling time and sample handling on the detection of *Staphylococcus aureus* in milk from quarters with subclinical mastitis. Can Vet J. 2002;43(1):38.

11. Bradley AJ, Leach KA, Breen JE, Green LE, Green MJ. Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Vet Rec. 2007;160(8):253-8.

12. Botrel MA, Haenni M, Morignat E, Sulpice P, Madec JY, Calavas D. Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. Foodborne Pathog Dis. 2010;7(5):479-87.

13. Sharma N, Singh NK, Bhadwal MS. Relationship of somatic cell count and mastitis: An overview. Asian-Australas J Anim Sci. 2011;24(3):429-38.

14. Idriss SE, Foltys V, Tančin V, Kirchnerová K, Zaujec K. Mastitis pathogens in milk of dairy cows in Slovakia. Slovak J Anim Sci. 2013;46(3):115-9.

15. San Martín B, Kruze J, Morales MA, Agüero H, León B, Esppinoza S, Iragüen D, et al. Bacterial resistance of mastitis pathogens isolated from dairy cows in the Vth Region, Metropolitan Region, and Xth Region, Chile. Arch Med Vet. 2002;34(2):221-34.

16. Pereira UP, Oliveira DG, Mesquita LR, Costa GM, Pereira LJ. Efficacy of *Staphylococcus aureus* vaccines for bovine mastitis: a systematic review. Vet Microbiol. 2011;148(2-4):117-24.

17. Schukken YH, Bronzo V, Locatelli C, Pollera C, Rota N, Casula A, et al. Efficacy of vaccination on *Staphylococcus aureus* and coagulase-negative staphylococci intramammary infection dynamics in 2 dairy herds. J Dairy Sci. 2014;97(8):5250-64.

18. Bradley AJ, Breen JE, Payne B, White V, Green MJ. 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(3):1706-20.

19. Middleton JR, Luby CD, Adams DS. Efficacy of vaccination against staphylococcal mastitis: a review and new data. Vet Microbiol. 2009;134(1-2):192-8.

20. Ismail ZB. Mastitis vaccines in dairy cows: Recent developments and recommendations of application. Vet World. 2017;10(9):1057-62.

21. Mella A, Ulloa F, Valdés I, Olivares N, Ceballos A, Kruze J. Evaluation of a new vaccine against *Staphylococcus aureus* mastitis in dairy herds of southern Chile. I. Challenge trial. Austral J Vet Sci. 2017;49(3):149-60.

22. Haskell SR, Loghry BL, BAS M, Wegner-Fowley K, Gilsenan VM, Johnson AL. Physical Exam. Large Animal Medicine for Veterinary Technicians. Eds: Loly, S., Hopkinson, H. John Wiley and Sons, Inc. 2021;29:67-103.

23. International Dairy Federation (IDF). Laboratory Methods for Use in Mastitis Work. Document 132. Brussels, Belgium 1981.

24. Schalm OW, Carroll EJ, Jain NC. Bovine Mastitis; Lea and Febiger: Philadelphia, PA, USA, p. 360. ISBN 0598052879; 1971; Lea and Febiger: Philadelphia, PA, USA, p. 360; 1971

25. Adkins PR, Middleton JR. Laboratory handbook on bovine mastitis. National Mastitis Council, Incorporated; 2017.

26. Hogan JS, Gonzalez RN, Harmon RJ, Nickerson SC, Oliver SP, Pankey JW, et al. Laboratory handbook on bovine mastitis. National Mastitis Council, Madison, WI. 1999;78(7):485-8.

27. Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC. Veterinary microbiology and microbial disease. 2nd Ed. Blackwell Science Ltd., UK; 2002.

28. International Dairy Federation (IDF). Enumeration of somatic cells. FIL-IDF Standard no. 148A. IDF, Brussels, Belgium. 1995.

Online ISSN: 2537-1045
Print ISSN: 1110-1423

29. Vasudevan P, Nair MK, Annamalai T, Venkitanarayanan KS. Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. Vet Microbiol. 2003;92(1-2):179-85.

30. Middleton JR, Ma J, Rinehart CL, Taylor VN, Luby CD, Steevens BJ. Efficacy of different Lysigin™ formulations in the prevention of *Staphylococcus aureus* intramammary infection in dairy heifers. J Dairy Res. 2006;73(1):10.

31. Luby CD, Middleton JR, Ma J, Rinehart CL, Bucklin S, Kohler C, et al. Characterization of the antibody isotype response in serum and milk of heifers vaccinated with a *Staphylococcus aureus* bacterin (LysiginTM). J Dairy Res. 2007;74(2):239-46.

32. Eisa AM, Abo El-Hassan DG, Samir A. Efficacy of a polyvalent mastitis vaccine against udder infection with *Staphylococcus aureus* in dairy cows in Egypt. Egypt.vet.med.Assoc. 2018; 78 (1) , 121 – 134.

33. Tenhagen BA, Edinger D, Baumgärtner B, Kalbe P, Klünder G, Heuwieser W. Efficacy of a Herd-Specific Vaccine Against *Staphylococcus aureus* to Prevent Post-Partum Mastitis in Dairy Heifers. J Vet Med A. 2001;48(10):601-7.

34. Landin H, Mörk MJ, Larsson M, Waller KP. Vaccination against *Staphylococcus aureus* mastitis in two Swedish dairy herds. Acta Vet Scand. 2015;57(1):1-6.

35. Nickerson SC, Owens WE, Tomita GM, Widel PW. Vaccinating dairy heifers with a *Staphylococcus aureus* bacterin reduces mastitis at calving. Large Anim Pract. 1999.
36. Ghobrial RF, EL Beskawy MA, EL Diasty MM, Farag VM, Eissa MI. Field Trial to Evaluate Vaccine and Antibiotic for Control of *Staph. aureus* Mastitis in Dairy cattle, Egypt. Alex J Vet Sci. 20181;56(1):88-94.

37. Leitner G, Yadlin N, Lubashevsy E, Ezra E, Glickman A, Chaffer M, et al. Development of a *Staphylococcus aureus* vaccine against mastitis in dairy cows. II. Field trial. Vet Immunol Immunopathol. 2003;93(3-4):153-8.

38. Dias RS, Fialho AC, Eller MR, Mantovani HC, da Silva CC, De Paula SO. Challenges in Vaccination against Mastitis (Doctoral dissertation, Ed (s) Berhardt LV, Advances in Medicine and Biology, p: 167-184. In book: Advances in Medicina and Biology) 2012.

39. Miller RH, Guidry AJ, Paape MJ, Dulin AM, Fulton LA. Relationship between immunoglobulin concentrations in milk and phagocytosis by bovine neutrophils. Am J Vet Res. 1988;49(1):42-5.

40. Smith GW, Lyman RL, Anderson KL. Efficacy of vaccination and antimicrobial treatment to eliminate chronic intramammary *Staphylococcus aureus* infections in dairy cattle. J Am Vet Med Assoc. 2006;228(3):422-5.

41. Hogan J, Smith KL. Coliform mastitis. Vet Res. 2003;34(5):507-19.

Online ISSN: 2537-1045
Print ISSN: 1110-1423

Table 1: The rate of clinical and subclinical mastitis before and after vaccination

Period of examination	Number of the examined animals (%)			
	Infected cows			Healthy cows
	Clinical mastitis	Subclinical mastitis	Total infected	
Before Lysigin	78 (13)	174 (29)	252 (42)	348 (58)
After Lysigin	40 (6.7)	110 (18.3)	150 (25)	450 (75)

Table 2: The rate of *S. aureus* and *E. coli* in the infected cows before and after vaccination

Period of examination	Number of infected cows (clinical and subclinical)	Number of bacterial isolates (%)	
		S. aureus	*E. coli*
Before Lysigin	252	63 (25)	78 (30.9)
After Lysigin	150	15 (10)	35 (23.3)

Online ISSN: 2537-1045
Print ISSN: 1110-1423

Table 3: The rate of somatic cell count (SSC) x 10^3/ml in chronically infected cows before and after vaccination

Cow number	SCC before Lysigin	SCC after Lysigin
1	669	380
2	>2000	951
3	>2000	924
4	1878	622
5	1414	671
6	931	690
7	1251	1204
8	>2000	1444
9	>2000	1360
10	953	618
11	>2000	815
12	979	338
13	308	300
14	>2000	1488
15	1818	539
16	>2000	380
17	>2000	>2000
18	>2000	361
19	1014	782
20	864	358
21	2000	500
22	2000	1000
23	702	290
24	1810	990
25	811	500

Online ISSN: 2537-1045
Print ISSN: 1110-1423

26	540	200
27	>2000	1640
28	1051	876
29	1055	776
30	1280	590
31	1818	695
32	1084	462
33	1638	900
34	1436	725
35	550	300

Table 4: Bulk milk tank SSC x 10^3 (BMTSSC) before and after vaccination

month	Bulk tank before Lysigin (2014)	Bulk tank after Lysigin (2015)	Bulk tank after Lysigin (2016)
January	718	300	220
February	650	280	215
March	630	270	210
April	581	270	210
May	560	260	220
June	550	265	210
July	425	240	215
August	450	245	230
September	432	234	220
October	440	230	180
November	520	250	190
December	600	200	200
Average SSC	546.33	234.5	210

Online ISSN: 2537-1045
Print ISSN: 1110-1423

Table 5: The number of bottles used for treatment of mastitis before and after vaccination

Type of medicine	Number of bottles used for treatment for 3 years		
	before Lysigin (2014)	after Lysigin (2015)	after Lysigin (2016)
Antibiotics	329	224	169
NSAIDs	154	105	75
Intramammary infusion	6733	5112	2746

Online ISSN: 2537-1045
Print ISSN: 1110-1423