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TESTING THE EFFICIENCY OF DIFFERENT TREATMENTS OF SUBCLINICAL *STAPHYLOCOCCUS AUREUS* MASTITIS IN COWS DURING THE DRY PERIOD

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Mastitis is still the most common disorder which is present in diary cows. Changes in genetics, nutrition and milking equipment affect the incidence of subclinical and clinical forms of mastitis. Staphylococcus aureus is the causative agent of subclinical and clinical forms of mastitis. In the acute form it can cause malignant mastitis in the form of granulomatous and necrotic changes. Chronic forms of staphylococcal mastitis often develop as subclinical changes.

Halting the entrance, the colonization and replication of the pathogen into the udder impose the constant need for regular milk controls and preventive and therapeutic measures in order to decrease the incidence of mastitis.

A modern approach in the eradication and control of mastitis is immunoprophilaxis, aimed towards the innovation of new vaccines against the most common causes of mastitis. In this study we have applied the vaccine prepared with S. aureus isolated from milk taken from the experimental farm and the referent capsular strain. The vaccine was applied twice two months before calving in a dose of 5 mL. The vaccine contained inactivated S. aureus JR3 bacterial cells in a quantity of 1 x 10^{10} cfu/mL and 5 mg SM capsule S. aureus 2286 strain. After vaccination of cows in late pregnancy, subclinical mastitis appears at a smaller frequency compared to the three experimental groups. To the first group of cows the antibiotic was applied intramammary, to the second group antibiotics were applied parenterally and intramammary and the third group served as the untreated control.

Key words: cows, immunoprophilaxis, mastitis, S. aureus, vaccine

INTRODUCTION

Mastitis is still the most common and most costly disorder on dairy farms. Changes in genetics, nutrition, milking equippment and husbandry affect the incidence of subclinical and clinical forms of mastitis.

Clinical forms can be peracute, acute, subacute and chronic. The most common form of mastitis is subclinical. The clinical form is however rarely found

and entails 3 – 5% of the herd at an annual level. Subclinical mastitis is diagnosed by the determination of pathogenic bacteria in milk samples taken from cows with no clinical signs of mastitis.

Inhibition of bacterial penetration into the udder imposes the need for constant preventive and therapeutic measures in order to reduce the incidence of mastitis. One of the important preventive measures is treatment of cows during the dry period. Such treatment includes intramammary application of antibiotics after the last milking which should provide the longest possible bacteria free time during the following lactation. Treatment during the dry period should be done with wide spectrum antibiotics. or with aimed drugs according to the antibiogram results (Hassan *et al*., 1999; Andrews, 2004).

Subclinical cases are routinely treated for *Streptococcus agalactiae* and *Staphylococcus aureus* while other bacteria are only identified. However, for the latter an antibiogram should be done and therapy with an adequate and prescribed antibiotic carried out (Soback, 2005). Further studies report the need for the use of the parenteral antibiotic Tylosine, two weeks before calving. This protocol for the prevention of mastitis has shown an increase in success rate from 74% to 91.6% after calving.

The aim of the antibiotic therapy is to destroy pathogens and at the same time not to cause any damage to the mammary gland. Subclinical *Streptococcus agalactiae* (*Str. agalactiae*) and *Staphylococcus aueus* (*S. aureus*) mastitis must be treated as soon as diagnosed.

Immunoprophilaxis and immunotherapy are aimed on the discovery of efficient vaccines for some of the most common microbial agents and they represent a modern approach to mastitis prevention. A number of published papers suggest that in order to improve the value of the vaccine with inactivated *S. aureus* (Middleton, 2008), alpha and beta toxoids (Chang, 2008), as well as parts of the bacterial capsule should be added (Calzolari, 1997; Watson, 1996; Opdebeeck, 1985). The monovalent vaccine against mastitis caused by *S. aureus* contains formalin inactivated *S. aureus* in the quantity of $1x10^{11}$ cfu/mL and 10 haemolitic units of alpha toxin (Han, 2000; Giraudo, 1997).

Literature data report that only formalin (0.5%) inactivated vaccines containing two strains of *S. aureus* are in use. One of the strains contains $1x10^{11}$ TBC (Total Bacterial Count) and the other $8.8x10^{10}$ TBC (Edinger, 2000). A vaccine containing two components, one bacterial cells 10^{10} microorganisms per milliliter (component C) and a toxoid (component T) (Watson, 1992; Nordhaug, 1994).

A completely novel approach in the immunization of the mammary gland was presented by O'Brien. He incorporated the *S. aureus* lysate into biodegradable particles which have the function to stimulate the production and opsonization of antibodies (O'Brien, 2001).

Results of the study described by Smith (2006) describe that a combination of vaccine and intramammary application of antibiotics result in a significant success in the therapy of chronic mastitis caused by *S. aureus*. Although all these vaccines indicate a substantial experimental success reflected in an increased serum antibody titre (but not in the milk), as well as decreased incidence in clinical and subclinical mastitis. It can be considered that the application of

immunoprophilaxis for treatment of udder inflammation is still a subject that needs further research.

MATERIAL AND METHODS

Isolation and identification of S. Aureus vaccinal strains

Parallel to strains (JR1, JR2, JR3 and JR4) the referent capsular strain of *S. aureus* 2286 was studied and included in the vaccine preparation. This pathogen does not require specific growth media. It grows well on blood agar containing 5 – 10% blood and forms regular, round, slightly convex colonies. Alongside the regular S forms, R and G colonies can be noted. Most often the colonies are pigmented golden-yellow or white. The colonies are about 3 – 5 mm in diameter and have a double alpha and beta hemolysis zone.

Vaccine preparation

The vaccine was prepared from the strain JR3 which displayed the most typical biochemical characteristics of *S. aureus* and the referent capsulated strain 2286.

Vaccine component A

A total of 5 L of Brain-Heart infusion was seeded with the isolated strain *S. aureus* JR3. The so inoculated medium was incubated at 37°C for 24 h. After this incubation period 0.4% vol/vol formalin was added and incubation continued for further 24h. After the microorganism was inactivated by formalin the culture was centrifuged at 7000 rpm at 4°C for 20 min. The success of the inactivation process was checked out by repeating the incubation of a sample on blood agar. Lack of bacterial growth indicated that inactivation was successful. After centrifugation the supernatant was discarded and the sediment resuspended in 0.9% NaCl at pH 7.0. The resuspended sediment represents the first constituent (A) of the vaccine.

Vaccine component B

The next step was the preparation of the referent capsular strain *S. aureus* 2286 which was seeded in 5 L of Brain-Heart infusion, as well. The so inoculated medium was incubated at 37°C for 24 h and thereafter centrifuged at 7000 rpm at 4°C for 20 min. The sediment was resuspended in saline and sterilized in an autoclave at 121°C, thus the referent strain *S. aureus* 2286 was inactivated. Subsequently, the sample was centrifuged under the same conditions as strain JR3. The obtained supernatant represents the second constituent (B) of the vaccine, consisting of capsules of the strain S. aureus 2286. In the supernatant 0.4% vol/vol formalin and 0.001% wt/vol of preservative timerasol were added.

The dose of the vaccine was 5 mL/cow, containing inactivated bacterial *S. aureus* JR3 cells in a quantity of $1x10^{10}$ cfu/mL (constituent A) and 5 mg SM capsule of strain *S. aureus* 2286 (constituent B).

By preparing the vaccine in the described manner a total of 135 doses (5 mL each) was obtained.

Vaccine sterility and toxicity tests

To test for sterility the vaccine was seeded into 2 test tubes containing serum broth, 2 test tubes containing nutritious broth, Jansen's media with added starch and Jansen media with glucose added, nutritious oblique agar and blood agar. In none of the listed media aerobic or anaerobic bacterial growth was recorded. This proved that the vaccine was sterile.

Toxicity was tested for on a total of 10 white mice, 2 guinea pigs, 4 sheep and 5 pregnant cows. The vaccine in a dose of 5mL was applied in two different ways i.e. there were formed two groups of 5 mice each. To one group the vaccine was applied intraperitoneally, and to the other group the route of application was subcutaneous. Mice to which the vaccine was applied intraperitoneally passed the biological test by not displaying any clinical signs. On *post mortem* examination there were no evident changes on the application site nor on parenchymatous organs. Mice to which the vaccine was applied subcutaneously behaved similarly, but showed on the application site small nodules.

Tests on guinea pigs and sheep did not indicate vaccine toxicity. The vaccine in a dose of 5 mL was applied subcutaneously in the neck region to pregnant cows, two months prior to calving. After vaccination there were no obvious changes in health status, feed intake, rumination or body temperature. On the application site a nodule the size of a nut was easy to palpate for a period of 10 - 20 days, after which it was reabsorbed. Thus, it was decided to administer the vaccine in two shots, 2.5 mL each, one on each side of the neck in the brachial region.

Cytology tests

The number of milk somatic cells was studied under a light microscope. Milk samples for cytology tests were prepared in the following fashion: on a microscope glass slide 0.01 mL of milk was smeared over a surface of 1 cm^2. To achieve this a piece of thin white cardboard with marked with a 1 cm x 1 cm square that was placed under the glass slide. After drying-up the sample at room temperature for 24h, fats from the smear were removed with xylol, dried up and fixed in ethanol for 5 minutes. Thereafter the samples were dried and dyed with the previously prepared dye.

The number of somatic cells was determined by a formula that defines the microscope factor (MF) and the average cell count in 5 field views:

$$MF = 100/r^2\pi \text{ x } n$$

MF – microscope factor; r – diameter of the eye field circle; π – 3.14; x – number of field viewings; n – average number of leucocytes counted.

Experimental design

In this study a total of 80 diary cows, positive for the presence of S. aureus were allotted into 4 experimental groups.

Experimental group 1. To the first group (n=20) to each cow an oily antibiotic benzatine cloxacilline suspension in a dose of 1000 mg/quarter (Dry cloxa-kel, Kela laboratories) was applied. The drug was applied after the last milking, two

months before expected calving. The first milk sampling was done before the application of the intramammary injector. The following was at delivery (colostrum) and thereon at 15 day intervals up to the development of subclinical and clinical signs of mastitis.

Experimental group 2. To the second group (n=20) the same oily antibiotic suspension was applied to each cow as in the first group. However, to the second group two weeks before calving tilozine (Tilozin 200®, Hemovet, Vršac) was applied via imtramuscular route for three days in a daily therapeutic dose of 10 mg/kg body mass. The first milk sampling was carried out before the application of the intramammmary suspension. The following sampling was done after delivery (colostrum sample) and thereon at 15 day intervals until first signs of subclinical or clinical mastitis.

Experimental group 3. To the third experimental group (n=20) the tested *S. aureus* mastitis vaccine was applied subcutaneously in the neck region two months before expected calving. Sampling was scheduled as for experimental groups 1 and 3.

Experimental group 4. Experimental group 4 was not treated with antibiotics nor was vaccinated. Sapling was scheduled as for the treated groups.

RESULTS

Microbiological tests of milk samples from experimental dairy cows suffering from clinical or subclinical mastitis

Microbiological tests were carried out on milk samples from all four experimental groups immediately prior to the dry period and preceding to vaccination and/or antibiotic treatment. Tests were carried out after calving at monthly intervals up to the manifestation of clinical and subclinical forms of mastitis in the vaccinated group of diary cows (Table 1). The trial lasted for a period of 8 months.

Table 1. Presence of *Staphylococcus aureus* before and after tested therapeutic treatments

Treated groups	No of cows	*S. aureus* at the start of the trial	Percentage %	*S.aureus* at the end of the trial	Percentage %
Vaccinated	20	8	40	3	15
Dry period intramammary therapy	20	5	25	7	35
Parenteral + intramammary therapy	20	7	35	5	25
Control	20	7	35	9	45

Out of the 20 tested diary cows at the beginning of the trial in the vaccinated group (experimental group 1) *S. aureus* was isolated in 8 (40%) animals. By the end of the experiment within this group only 3 (15%) tested positive. In the group receiving treatment during the dry period (experimental group 2) *S. aureus* was isolated at the start in 5 (25%) and at the end of the trial in 7 (35%) animals. Experimental group 3 had results somewhat similar to group 2 as at the beginning of the trial 7 (35%) cows tested *S. aureus* positive and by the end 5 (25%) tested negative. Group 4, which served as the control group as was left untreated throughout the experiment had the following results: at the start of the trial 7 (35%) cows tested *S. aureus* positive and at the end of the testing period a total of 9 (45%) animals were positive.

Table 2. Presence of subclinical and clinical forms of mastitis caused by *Staphylococcus aureus* in the tested groups

Treated groups	No of cows	Subclinical mastitis	Percentage %	Clinical mastitis	Percentage %
Vaccinated	20	3	15	0	0
Dry period intramammary therapy	20	4	20	3	25
Parenteral + intramammary therapy	20	3	15	2	10
Control	20	5	25	4	20

Studies on the presence of subclinical and clinical forms of mastitis have shown that subclinical forms within the vaccinated group were present in 3 (15%) cases and clinical forms were absent. In Group 2 subclinical mastitis was present in 4 (20%) cows and the clinical form was present in 3 (15%) animals. The group of diary cows treated during the dry period with i.m. Tilozin 200 two weeks before delivery subclinical mastitis was present in 3 (15%) cows and the clinical form was recorded in 2 (10%) cows. In the control untreated group (Experimental group 4) subclinical mastitis was present in 5 (25%) and clinical mastitis in 4 (20%) diary cows.

Somatic cells count results in the experimental groups of cows affected by subclinical and clinical forms of mastitis

Table 3 shows the results of the somatic cells count (SCC) for all vaccinated groups. The average number of somatic cells for the vaccinated cows was 521 962.1 ± 44 306.58. It is cleat that in these groups the number of somatic cells decreased from the starting referent value of 100% down to 61.64% by the end of the trial. Table 3 indicates that in the second month of the experiment i.e. the colostral period, the number of somatic cells increased by 13.3%.

Table 3. Number of somatic cells present in the milk of vaccinated dairy cows

	Month 0 – dry period	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7
Σ 1-20	14272900	16181800	8391000	7467300	5917700	12046800	8797200
$\bar{X}$	713645	809090	419550	373365	295885	602340	439860
SD	49615.32	54171.12	33822.03	39239.73	21924.17	45814.11	37783.19
$\bar{X}$	100	113.37	58.79	52.32	41.46	84.40	61.64

Table 4. shows the results of the somatic cells count (SCC) for all cows receiving treatment during the dry period. The average number of somatic cells for the treated cows was 461 550.5 ± 35 534.54. It is clear that in this group the number of somatic cells decreased from the starting referent value of 100% down to 49.93% by the end of the trial. After calving the percentage of SCC was 82.5% of the starting value immediately before the dry period.

Table 4. Number of somatic cells present in the milk of dairy cows treated during the dry period

	Month 0 – dry period	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7
Σ 1-20	15191800	12543800	9220200	5321200	6220670	8534000	7585400
$\bar{X}$	759590	627190	461010	266060	311033.5	426700	379270
SD	31470.35	39574.92	37162.83	27181.98	21052.85	28809.59	36596.78
%	100	82.56954	60.69195	35.02679	40.94755	56.17504	49.93088

Table 5. Number of somatic cells in the milk samples taken from diary cows treated intramammary and with i.m. antibiotics during the dry period

	Month 0 – dry period	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7
Σ 1-20	17663600	13838100	8532600	6466870	5636500	4851600	5374202
$\bar{X}$	883180	691905	426630	323343.5	281825	242580	268710,1
SD	31470.35	39574.92	37162.83	27181.98	21052.85	28809.59	36596,77
%	100	78.34247	48.30612	36.61128	31.91026	27.46665	30.42529

Table 5 depicts the number of somatic cells (SCC) in all diary cows treated with parenteral antibiotics two weeks before calving. The average SCC was 445 453.7 ± 31 692.75. It can be seen that in this group of animals the SCC decreased from the initial referent value of 100% down to 30.42%. Immediately after delivery the number of somatic cells decreased to 78.3% from the starting value.

Table 6 describes the SCC results for the untreated control group. The average SCC value for the control group was 694 629.30 ± 56 773.46. At the end of the studied seven month period SCC declined to 26.76% of the starting value. In the peripartal period (colostral period) the SCC was 47.6% of the starting value.

Table 6.Number of somatic cells present in the milk of control dairy cows

	Month 0 – dry period	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7
Σ 1-20	33340600	15893000	11457440	10699600	8133600	8799800	8922800
$\bar{X}$	1667030	794650	572872	534980	406680	439990	446140
SD	189055.74	49548.62	46991.97	29180.11	24367.77	30881.91	27388.13
%	100	47.66861	34.36483	32.0918	24.39548	26.39365	26.76257

Table 7 illustrates the statistical significance of the difference in the number of somatic cells present in milk samples of vaccinated and control diary cows, as well as differences between vaccinated cows and cows treated during the dry period. There is a significant statistical difference between the vaccinated group and the group receiving antibiotics two weeks before calving.

Table 7. Variance analysis for treated cows (ANOVA) $F = 5.676$, $p \leq 0.01$

Group	Difference of means	q	p
Control / vaccinated	172700	3.608	$p \geq 0.05$
Control / dry period therapy	233100	4.87	$p \leq 0.01$
Control / parenteral therapy	249200	5.207	$p \leq 0.01$
Vaccinated / dry period therapy	60410	1.262	$p \geq 0.05$
Vaccinated / parenteral therapy	76510	1.599	$p \geq 0.05$
Dry period therapy / parenteral therapy	16100	0.3364	$p \geq 0.05$

Table 8 clearly shows the mean, minimal and maximal SCC values during the dry period for all experimental groups. The same parameters are given for the postpartal i.e. colostral period in Table 9.

Table 8. Mean, minimal and maximal SCC values during the dry period

Stat. param.	Vaccinated	Control	Dry period therapy	Parenteral therapy
$\bar{X}$	713645	1667030	759590	883180
Maximum	1696000	2048000	1513600	1760000
Minimum	88100	51200	88000	32800
SD	49615.32	60155.71	31470.34	52249.99

Table 9. Mean, minimal and maximal SCC values during the colostral period

Stat. param.	Vaccinated	Control	Dry period therapy	Parenteral therapy
$\bar{X}$	809090	794650	627190	691905
Maximum	1872000	1760000	1430000	2240000
Minimum	97600	108800	97600	65500
SD	54171.12	49548.62	39574.92	60197.21

DISCUSSION

According to the report of Stojanovic (2001) mastitis is still one of the most expensive illnessess of diary cattle. It is estimated that at least 40% of cows are infected with at least one of the agents which cause mastitis, but only 2 – 3% suffer from the clinical form of mastitis.

Subclinical forms are mainly caused by *S. agalactiae, Streptococcus* spp., *S. aureus* and *Staphylococcus* spp. are common and represent 35 – 40% of all subclinical forms of mastitis in diary cows. However, Nordhaug (1994) and Edinger (2000) observed 16% cows to be suffering from *S. aureus* subclinical mastitis and Giraudo (1997) reports 19% affected cows. Our results show a higher incidence of subclinical forms of mastitis in the studied herds reaching a value of 25%. Nordhaug (1994) describes *S. aureus* subclinical mastitis in 16% control and 8.6% vaccinated cows. Much higher values for the control and vaccinated groups (43.0% and 35.4%, respectively) were obtained by Watson (1996). In our experiment we have determined 25% control diary cows and 15% vaccinated cows to be positive for *S. aureus* mastitis compared to *S. aureus* subclinical mastitis at the very start of the trial i.e. before vaccination.

Biggs (2002) in his work shows the advantage of tylozine parenteral therapy two weeks before expected calving compared to the classical intramammary therapy during the dry period. His results indicate that cows receiving two weeks before calving 30 mL tylozine for three consecutive days increased the *S. aureus*

mastitis therapy success rate from 68% to 88%. Our results illustrate that after parenteral application of tylozine the number of intramammary infections caused by *S. aureus* changed from 7 (35%) at the beginning of the study to 5 (25%) registered at the end of the trial. These results coincide with the findings reported by Biggs (2002).

The standard therapy during the dry period presupposes the intramammary application of benzatine cloxacilline. However, it has shown a poor response compared to parenteral application of tylozine. Our work has shown similar results to Shephard (2005) who describes an insignificant drop in *S. aureus* subclinical mastitis after calving in cases where during the dry period therapy consisted of cloxacilline and cephalonine. On the other hand Hassan (1999) has applied cloxacilline during the dry period and thus obtained a positive outcome.

Based upon the obtained results we are of the opinion that vaccination against *S. aureus* would give even better results if combined with parenteral antibiotics two weeks before calving and if diary cows were revaccinated one month before expected delivery.

The low incidence of clinical forms of mastitis caused by *S. aureus* published by Edinger (2000) was described to be present in 3.8% in the control group and 2.4% in the vaccinated group of diary cows. A much higher frequency of clinical mastitis was described by Watson (1996), and was 34.5% for the control and 16.3 for vaccinated animals. The above values differ greatly from those described by Nordaug (1994) who states the rate of clinical mastitis to be 6% and no cases in the vaccinated group.

Our results are closest to those described by Nordhaug, as we did not register a single case of clinical mastitis in the vaccinated group while in the control group were affected 14.2% cows. These results clearly indicate the possibility to decrease the frequency of clinical forms of mastitis by vaccination. In the non vaccinated groups cases of clinical mastitis were still present. Diary cows receiving the antibiotic Tylozine the number of registered cases was 2 (10%) and in the group of cows receiving during the dry period an intramammary application of antibiotics there were 3 (15%) registered cases. Shephard (2005) reports the postpartal presence of clinical forms of mastitis after an intramammary cloxacilline application during the dry period. Opposite results were published by Digwell (2003) who reports a significant decrease of cases of clinical mastitis in animals receiving an intramammary application of cloxacilline and 1.5 g tylomicozine in the post partal period. Cows receiving cloxacilline decreased udder infections from 62% down to 56.9%, while cows receiving tylomicozine decreased the occurrence of mastitis by a half.

During the involution of the mammary gland the number of somatic cells (SCC) increases to 1 000 000 cells/mL, probably as a consequence of stopped milking. However, prior to delivery SCC returned to normal values (Nickerson, 1989).

Our study has confirmed that the average SCC at the very start of the dry period was within the range of previously published data between 713 645 and 1 667 030.

At the start of lactation the SCC can increase up to 2 500 000 cells/mL. In our study the average SCC at the start of lactation (immediately after delivery) ranged from 627 190 to 809 090. The maximal SCC values were from 1 430 000 to 2 240 000, and the minimal values were in the range from 65 500 to 108 800. The reported results clearly indicate that there is an increase of somatic cells in the control group compared to the treated groups. These results are not compatible to the results published by Edinger (2000), Giraudo (1997), Hoedmaker (1999), who did not describe a significant SCC increase between the tested groups.

When we have indexed the values of SCC and when the starting value was determined as 100%, we recorded a smaller drop in SCC in the vaccinated group compared to the other groups. SCC in the control group decrease from the starting value of 100% by 26.76%. Cows treated with intramammary therapy during the dry period decreased by 49.90% and for cows receiving parenteral antibiotic therapy SCC decreased by 30.42%. SCC for the vaccinated group was higher compared to the other experimental groups and decreased from the starting value of 100% to 61.10% which can be explained by vaccination.

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ISPITIVANJE EFIKASNOSTI RAZLIČITIH TRETMANA SUBKLINIČKIH MASTITISA KRAVA IZAZVANIH *STAPHYLOCOCCUS AUREUS*-OM U ZASUŠNOM PERIODU

VAKANJAC SLOBODANKA, PAVLOVIĆ M i PAVLOVIĆ V

SADRŽAJ

Mastitis je još uvek najučestalije i "najskuplje" obolenje na mlečnim farmama. Promene u genetici, ishrani, aparatima za mužu i načinu držanja krava utiču na učestalost pojave subkliničkih i kliničkih mastitisa. *Staphylococcus aureus* izaziva subkliničke i kliničke forme mastitisa, koje mogu u akutnoj formi da izazovu teške, maligne mastitise u vidu granulaomatoznih i nekrotičnih promena. Hronične forme stafilokoknog mastitisa uglavnom prolaze kao subklinički oblici obolenja mlečne žlezde. Sprečavanje prodora patogenog uzročnika u mlečnu žlezdu, njegovo naseljavanje i razmnožavanje, nameću stalnu potrebu za redovnim kontrolama mleka kao i preduzimanje preventivnih i terapijskih mera u cilju smanjenja nastanka mastitisa. Moderan pristup suzbijanju i kontroli mastitisa je imnunoprofilaksa koja je usmerena na pronalaženje efikasnih vakcina protiv nekih

najčešćih uzročnika mastitisa. U našem radu koristili smo autohtonu vakcinu koju smo pripremili od *S. aureus*-a izolovanog iz mleka uzetog sa ogledne farme i referentnog kapsularnog soja *S. aureus*. Vakcina je dvokratno aplikovana oglednim kravama dva meseca pred telenje u dozi od 5 ml, a sastojala se od inaktivisanih bakterijskih ćelija *S. aureus* JR3 u količini od $1x10^{10}$ cfu/ml i 5 mg SM kapsule soja *S. aureus* 2286. Nakon dvokratne aplikacije ispitivane vakcine u visokom graviditetu, subklinički mastitisi krava su se pojavljivali u značajno manjem procentu, u odnosu na ostale tri ogledne grupe. Pri tome je jednoj oglednoj grupi krava bio aplikovan antibiotik intramamarno dok su životinjama druge grupe antibiotici bili aplikovani i parenteralno i intramamarno. Treća grupa se sastojala od plotkinja koje nisu bile podvrgnute ni jednom od navedenih tretmana.