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Commercial Mastitis Vaccines - Effects on Mastitis Rates and Somatic Cell Counts in Saanen Goats

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ABSTRACT

***Background*:** *Staphylococcus* spp. are the most frequently isolated microorganisms in mastitis cases of small ruminants. The virulence factors of *Staphylococcus* spp. are critical in the treatment. Therefore, preventive medicine and mastitis control programs, especially herd vaccinations are of great importance in the prevention of mastitis. However, it is not always easy to obtain these vaccines under field conditions. This study, it was aimed to compare the effects of different commercial *Staphylococcus* spp. vaccines licensed for bovines and species-specific mastitis vaccines on mastitis rates and somatic cell count (SCC) on Saanen goats on field conditions.

***Materials, Methods & Results*:** The animal material consisted of 115 (230 udder halves) nulliparous Saanen goats. Goats were randomly grouped as bovine vaccine 1 (BV_1, n = 58), bovine vaccine 2 (BV_2, n = 58), small ruminant vaccine (SRV, n = 56), and control (n = 56). Vaccines were administered to goats in 2 doses according to the label regimen. First milk samples were collected between 0-5 days in milk (DIM) for microbiological analysis and 25-35 DIM for SCC. The other milk samples were collected at 25-35 (1st month) DIM, 60-65 (2nd month) DIM, 85-95 (3rd month) DIM, 115-125 (4th month) DIM, 145-155 (5th month) DIM for microbiological analysis and SCC. Non-*aureus* staphylococci (NAS) and *Staphylococcus aureus* were the most frequently isolated microorganism. It was found that the total mastitis rate decreased in vaccine groups compared to the control group. A significant difference was found only in the BV_2 and SRV groups. The significant difference in *S. aureus* infection was found only in the SRV group. Mastitis vaccines used in this study decreased the *NAS* mastitis rate, but no significant difference was observed. It was found that the clinical mastitis incidence decreased in all vaccine groups compared to the control group, and a significant difference was found between the BV_2 and SRV groups compared to the control group ($P < 0.05$). Somatic cell count was lower in the SRV and BV_2 groups compared to the control group ($P < 0.05$).

***Discussion*:** In this study, compatible with the previous reports NAS and *S. aureus* were the most frequently isolated microorganism. The diversity of virulence factors of *Staphylococcus* spp. also plays an important role in its high incidence. In some countries, mastitis vaccines used in cows are also administered to small ruminants for reducing infection rates. Similarly, in this study, it was found that the mastitis rate decreased in all vaccine groups compared to the control group. A significant difference was found only in the BV_2 and SRV groups. It is thought that the reason for the statistical difference may be due to the biofilm antigen in the BV_2 and SRV. In addition, J5 strain in the BV_2 is estimated to be effective in reducing the prevalence of gram-negative mastitis. It was observed that the infection rates decreased in the vaccine groups, especially due to *S. aureus* and NAS. Spontaneous treatment rates were very close to each other between the groups. The reason for the high rate of spontaneous treatment in this study can be explained by the fact that the animals were young and in their 1st lactation. SCC was lower in all vaccine groups compared to the control group. This situation is associated with the decrease in infection rates related to the use of vaccines. It was observed that SCC was lower in the vaccine groups. In addition, SCC was found to be lower in this study compared to similar studies. However, it is evident that the use of species-specific vaccines in the SRV group significantly reduced the rates of total *S. aureus* mastitis, subclinical NAS mastitis, and new infections by NAS compared to other vaccines. Furthermore, the species-specific vaccine significantly increased the rate of spontaneous treatment for *S. aureus* mastitis.

Keywords: field condition, mastitis, milk, Non-*aureus* staphylococci, small ruminant, vaccine.

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INTRODUCTION

Mastitis is a cause of significant economic losses in dairy ruminants, not only because of the decrease in milk yield and quality but also because of the increase in the number of culled animals and treatment costs [1,7,12,33]. There are many studies on mastitis prevalence and the most isolated microorganisms in sheep and goats are non-*aureus* staphylococci (NAS, formerly known as coagulase-negative *Staphylococcus*) and *S. aureus* [1,8,15].

Vaccination can play an important role in mastitis control programs. There are different vaccines commercially available against *S. aureus*. In general, commercial vaccines are bacterial-based vaccines that contain inactive *S. aureus* strains [27].

In recent studies, it was observed that biofilm structures play a role in *S. aureus* mastitis etiology [35,39]. Biofilm is highly resistant to immune cells, especially phagocytosis of macrophage, the bactericidal action of antibiotics and disinfectants [5,10]. For this reason, it is known that commercial vaccines contain biofilm antigens in recent years.

This study, it was aimed to compare the effects of different commercial *Staphylococcus* spp. vaccines licensed for bovines and species-specific mastitis vaccines on different mastitis rates and Somatic Cell Count (SCC) on Saanen goats on field conditions.

MATERIALS AND METHODS

Animals

The study was performed in 2 different commercial dairy Saanen herds locations (37° 25'09.4 "N 30° 26'12.9" E - 37° 11'14.2 "N 30° 21'40.9" E) in Turkey. The animal material consists of 115 nulliparous Saanen goats (230 udder halves), between 1-2 years of age without any health and mammary problems. Goats had synchronized oestrus and gave birth in an average of 5 ± 0.12 days. All goats were fed *ad libitum* with a balanced diet. All lactating goats were milked twice a day at 06:00 and 18:00 throughout the lactation period. Milking hygiene included pre-and post-milking sanitization of teats.

Commercial mastitis vaccines and groups

The mastitis vaccines used in the study were determined as follows: Lysigin® vaccine[1] (licensed for cattle), Startvac® vaccine[2] (licensed for cattle), and Vimco® vaccine[2] (licensed for small ruminants).

- Lysigin® vaccine contains 5 different phages and 5, 8, 336 capsules of *S. aureus* serotype (bovine vaccine$_1$, BV$_1$).

- Startvac® vaccine is an inactivated and polyvalent vaccine containing inactivated *Escherichia coli* J5, inactivated *S. aureus* capsular polysaccharide 8 (SP 140 strain), and a biofilm-associated antigenic complex (bovine vaccine$_2$, BV$_2$).

- Vimco® vaccine contains inactivated *S. aureus*, a biofilm-associated antigenic complex (small ruminant vaccine, SRV).

Goats were randomly grouped as BV$_1$ (n = 58), BV$_2$ (n = 58), SRV (n = 56), and control group (n = 56). Vaccines were administered to goats in 2 doses according to the label regimen. Licensed vaccine doses in cattle were determined according to Kautz *et al*. [20].

Milk sample collection

During this study, milk samples were collected monthly from a total of 230 udder halves for 5 months. First milk samples were collected between 0-5 DIM (days in milk) for microbiological analysis and 25-35 DIM for SCC. The other milk samples were collected at 25-35 (1st month) DIM, 60-65 (2nd month) DIM, 85-95 (3rd month) DIM, 115-125 (4th month) DIM, 145-155 (5th month) DIM for microbiological analysis and SCC. All milk samples were collected from each udder halves into sterile tubes[3] (Eurotubo Deltalab, no: 429946) during morning milking [34]. All collected milk samples were maintained at or below 4°C during transport to the laboratory for analysis within 2 h.

Microbiological analysis and milk somatic cell count

Microbiological analysis and somatic cell counting were carried out using the standard milk sample examination techniques, which exceeded the standard recommended by the International Dairy Federation [16]. Ten microliters of milk sample were single inoculated onto different agars[4] (Sheep blood agar, code: CM0271; MacConkey agar, code: PO0149). The same samples were double inoculated onto Sabouraud dextrose agar[4] with chloramphenicol supplement (code: PO0192). Single inoculated and one of the double inoculated plates were incubated for 24-48 h at 37°C in an aerobic environment and another one of the double inoculated plates was incubated at room temperature. The colonies were identified according to gram staining, hemolysis, catalase tests, and coagulase properties. The morphology of the bacterial colonies

obtained was checked for colony size, shape, texture, and color. Hemolysis of the red blood cells in the sheep blood agar was also checked for by identification of the changes in the media around and under the colonies. After 48 h, plates with no growth were recorded as no growth. Plates with mixed growth were subcultured to obtain pure colonies [24]. Organisms were identified and quantified using standard laboratory techniques [28]. The SCC was determined using the Fossomatic method[5] according to the International Dairy Federation [17].

Calculation of mastitis rates

$$\text{Mastitis rate: } \frac{\text{The number of infected udder halves}}{\text{The number of all udder halves}}$$

$$\text{New infection rate: } \frac{\text{The number of newly infected udder halves in the group}}{\text{The number of all udder halves in the same group}}$$

New infection refers to the udder half that is microbiologically negative in the 1st sampling but microbiologically positive in the next sampling. In addition, microorganism-positive udder halves at 0-5 DIM were defined as a new infection.

$$\text{Chronic infection rate: } \frac{\text{The number of chronically infected udder halves in the group}}{\text{The number of all udder halves in the same group}}$$

Chronic infection was defined as microbiologically positive udder halves in at least 2 consecutive milk samples.

Spontaneous treatment was defined as being microbiologically positive on the 1st sampling but microbiologically negative on 2 subsequent consecutive samplings for at least 3 milk sampling periods.

Statistical analysis

Data were collected and initially analyzed using Excel and Access[6] 365 and Minitab[7] 16.1 program. Descriptive and graphical analyses were carried out to explore the data. Chi-square statistical analysis was used to evaluate parameters such as mastitis incidence, new and chronic infection rates, and spontaneous treatment rates. The SCC was $\log_{10}$ transformed before analysis. One way repeated measures analysis of variance (ANOVA) procedure was used when analyzing the differences between groups in terms of SCC. The results are presented as mean ± standard error (X ± SE). If $P < 0.05$, it was considered statistically significant.

RESULTS

Non-*aureus* staphylococci (52.26%), *Staphylococcus aureus* (26.85%), *Streptococcus* spp. (4.17%), *Klebsiella* spp. (3.70%), *Shigella* spp. (1.85%), *Corynebacterium* spp. (1.39%), *Candida* spp. (1.39%), *Escherichia coli* (0.93%) and *Bacillus* spp. (0.46%) were found among the total microorganisms isolated during the study.

Total mastitis rate was lower in the vaccine groups compared to the control group. There was no significant difference between the vaccine and control groups in terms of total *NAS* mastitis incidence ($P > 0.05$). There was a statistical difference between the vaccine and control groups in terms of the total *S. aureus* mastitis incidence only in the SRV group ($P < 0.01$). Mastitis incidences according to *NAS* and *S. aureus*, and all microorganisms are shown in Table 1.

It was observed that clinical and subclinical mastitis rates decreased in the vaccine groups compared to the control group. *Staphylococcus* spp. and *Klebsiella* spp. are isolated at a higher rate compared to other microorganisms in clinical mastitis. *NAS* and *S. aureus* are the most isolated microorganism in subclinical mastitis. Subclinical and clinical mastitis incidences according to NAS, *S. aureus*, and all microorganisms are shown in Table 2.

Gram-negative mastitis agents were not detected in the BV_2 group. The highest rate was observed in the BV_1 group. It was observed that the gram-negative mastitis decreased in the BV_2 group compared to the control and BV_1 groups ($P < 0.05$). It is observed that the rates of new infections in SRV and BV_2 groups decreased significantly ($P < 0.05$). The new infection caused by NAS and *S. aureus* infections was lower in the vaccine group. The new infection rate according to NAS, *S. aureus*, and all microorganisms are shown in Table 3.

The group with the lowest rate of chronic mastitis is the BV_1 group and the group with the highest rate is the SRV group. The spontaneous treatment rates were close to each other but decreased in the control group compared to the vaccine groups. Chronic mastitis and spontaneous treatment rates according to NAS, *S. aureus*, and all microorganisms are shown in Table 4. The mean of SCC by months and groups is shown in Table 5.

Table 1. Mastitis rates according to sampling days.

	Control	BV_1	BV_2	SRV	*P* value
0-5 days mastitis	0.155	0.052	0.125	0.103	NS
25-35 days mastitis	0.431^{a}	$0.276^{a,b}$	0.232^{b}	0.224^{b}	$P < 0.05$
55-65 days mastitis	0.185	0.103	0.071	0.086	NS
85-95 days mastitis	0.250^{a}	$0.190^{a,b}$	0.089^{b}	$0.138^{a,b}$	$P < 0.05$
115-125 days mastitis	0.148	0.172	0.074	0.103	NS
145-155 days mastitis	0.148	0.196	0.148	0.121	NS
Total mastitis	0.221^{a}	$0.165^{a,b}$	0.123^{b}	0.129^{b}	$P < 0.01$
Total NAS mastitis	0.121	0.098	0.078	0.080	NS
Total *S. aureus* mastitis	0.070^{a}	$0.037^{a,b}$	$0.039^{a,b}$	0.026^{b}	$P < 0.01$

Statistical analysis was evaluated between the lines. Statistical differences between groups were indicated with different letters (a,b). Statistical significance was determined as $P < 0.05$. NS: not significant.

Table 2. Subclinical and clinical mastitis rates according to sampling days.

	Control	BV_1	BV_2	SRV	*P* value
0-5 days subclinical mastitis	0.138	0.052	0.125	0.103	NS
25-35 days subclinical mastitis	0.362	0.276	0.214	0.207	NS
55-65 days subclinical mastitis	0.167	0.086	0.071	0.069	NS
85-95 days subclinical mastitis	0.231	0.155	0.089	0.138	NS
115-125 days subclinical mastitis	0.130	0.138	0.074	0.103	NS
145-155 days subclinical mastitis	0.148	0.196	0.148	0.121	NS
Total subclinical mastitis	0.197^{a}	$0.150^{a,b}$	0.120^{b}	0.124^{b}	$P < 0.01$
Total clinical mastitis	0.024^{a}	$0.014^{a,b}$	0.003^{b}	0.006^{b}	$P < 0.01$
Subclinical NAS mastitis	0.091^{a}	0.075^{ab}	$0.063^{a,b}$	0.049^{b}	$P < 0.05$
Subclinical *S. aureus* mastitis	0.048	0.035	0.024	0.023	NS

Statistical analysis was evaluated between the lines. Statistical differences between groups were indicated with different letters (a,b). Statistical significance was determined as $P < 0.05$. NS: not significant.

Table 3. New infection rates according to sampling days

	Control	BV_1	BV_2	SRV	*P* value
0-5 days new infection	0.155	0.052	0.125	0.103	NS
25-35 days new infection	0.362^{a}	$0.259^{a,b}$	0.179^{b}	$0.207^{a,b}$	$P < 0.05$
55-65 days new infection	0.056^{a}	0.103^{a}	0.071^{a}	0.086^{a}	NS
85-95 days new infection	0.037^{a}	$0.103^{a,b}$	0.071^{b}	$0.034^{a,b}$	$P < 0.05$
115-125 days new infection	$0.130^{a,b}$	0.155^{a}	0.037^{b}	$0.069^{a,b}$	$P < 0.05$
145-155 days new infection	0.130	0.125	0.111	0.052	NS
Total new infection	0.170^{a}	$0.136^{a,b}$	0.093^{b}	0.092^{b}	$P < 0.01$
New infection by NAS	0.091^{a}	0.075^{ab}	$0.063^{a,b}$	0.049^{b}	$P < 0.05$
New infection by *S. aureus*	0.048	0.035	0.024	0.023	NS

Statistical analysis was evaluated between the lines. Statistical differences between groups were indicated with different letters (a, b, c...). Statistical significance was determined as $P < 0.05$. NS:not significant

Table 4. Chronic mastitis and spontaneous treatment rates according to sampling days.

	Control	BV_1	BV_2	SRV	*P* value
Chronic mastitis by NAS	0.225	0.176	0.192	0.321	NS
Chronic mastitis by *S. aureus*	0.217	0.077	0.308	0.111	NS
Total chronic mastitis	0.219	0.140	0.220	0.222	NS
NAS spontaneous treatment	0.432	0.412	0.500	0.333	NS
S. aureus spontaneous treatment	0.400^{a}	$0.538^{a,b}$	$0.500^{a,b}$	0.889^{b}	$P < 0.05$
Total spontaneous treatment	0.477	0.500	0.525	0.558	NS

Statistical analysis was evaluated between the lines. Statistical differences between groups were indicated with different letters (a,b). Statistical significance was determined as $P < 0.05$. NS: not significant.

Table 5. Somatic cell count (SCC) x 10^3 according to sampling days.

	Control	BV_1	BV_2	SRV	*P* value
25-35 days SCC	815 ± 146	823 ± 115	695 ± 105	607 ± 101	NS
55-65 days SCC	1282 ± 156^{a}	818 ± 118^{b}	852 ± 116^{b}	819 ± 119^{b}	$P < 0.05$
85-95 days SCC	1201 ± 146	904 ± 121	768 ± 107	1016 ± 151	NS
115-125 days SCC	835 ± 103	958 ± 122	651 ± 76	681 ± 86	NS
145-155 days SCC	887 ± 127	887 ± 125	752 ± 134	582 ± 60	NS
Total SCC	1012.1 ± 62.6^{a}	$877.9 \pm 53.5^{a,b}$	746.8 ± 48.7^{b}	$743.4 \pm 49.1^{b*}$	$P < 0.05$ $P < 0.01$

Statistical analysis was evaluated between the lines. Statistical differences between groups were indicated with different letters (a,b). Statistical significance was determined as $P < 0.05$. $^{*}P < 0.01$. NS: not significant.

DISCUSSION

Many studies on mastitis prevalence and microorganism isolation have been performed in goats so far [1,8,15]. It was reported that NAS is the most common microorganism for intramammary infections in 7.192 goat milk samples [9]. Similarly, it was reported that the most isolated microorganisms in goats were NAS (12.3%) and *Staphylococcus aureus* (1.2%), while the prevalence of other bacteria was less than 0.5% [32]. Furthermore, the rate of *Staphylococcus* spp. was 73.5% in all isolates in the Sardinia region of Italy. In this study, compatible with the previous reports NAS and *S. aureus* were the most frequently isolated microorganism. According to these study data, *Staphylococcus* spp. has a high prevalence, and advanced protection and control measures are required for this microorganism. It is stated that the prevalence of NAS is very high due to contagious transmission and causing subclinical chronic infections [18]. In addition, the subclinical form of infection, the fact that the causative agent can not be noticed in milk and udder by the milker, plays a role in the continuous increase in the prevalence of NAS. The diversity of virulence factors of *S. aureus* also plays an important role in its high incidence [19,31].

The most frequently isolated gram-negative bacteria species, according to previous studies, were *E. coli*, *Pseudomonas* spp., *Citrobacter* spp., and *Klebsiella* spp. [13,41]. Farm hygiene and environmental factors have a close relationship with gram-negative microorganisms [14]. Although *Klebsiella* spp. was the most isolated gram-negative agent in this study, it was isolated at a very low rate compared to all microorganisms. It is estimated that frequent cleaning of milking equipment, maximum attention to milking hygiene, and udder teat dipping treatment significantly reduce the prevalence of these infections in this study.

The use of licensed commercial vaccines in sheep and goats in the world is limited. In some countries, mastitis vaccines used in cows are also administered to small ruminants for reducing infection rates. In a study, Lysigin® vaccine was administered to Saanen goats. It has been found that the infection rate was 1.64 (infection/goat) in the vaccinated group and 2.67 (infection/goat) in the control group. It has been noted that the Lysigin® vaccine

reduced infection rates ($P < 0.122$) [20]. In another study, Masti-Vac® vaccine[8] was administered and it was reported that the mastitis rate in the vaccine and control group was 1.3% and 2.7%, respectively [22]. Some researchers used a herd-specific vaccine in cows. It has been found that the mastitis rate was lowest in the vaccinated group ($P > 0.05$) [37]. Similarly, in this study, it was found that the mastitis rate decreased in vaccine groups compared to the control group. A significant difference was found only in the BV_2 and SRV groups. It is believed that the reason for the statistical difference may be attributed to the biofilm antigen in BV_2 and SRV. Considering the significant role of *Staphylococcus* species' biofilm virulence, it can be said that vaccines provide more effective protection. Additionally, it is estimated that the J5 strain in BV_2 has some effectiveness in reducing the prevalence of gram-negative mastitis.

In the study investigating the efficacy of the Mastivac1® vaccine[9], which consists of different *S. aureus* isolates, on experimental mastitis, the infection rates (35.3% - 90.5%) were found to be statistically significant in the vaccine and control groups, respectively ($P < 0.01$) [23]. The Vimco® vaccine was used by researchers, and it was stated that NAS and *S. aureus* mastitis rates decreased effectively in the vaccine group ($P < 0.001$) [38]. The results of the previous study align with the findings of this study. Unlike previous studies, the prevalence of *S. aureus* mastitis in the BV_1 group was not statistically significant. The statistical difference in *S. aureus* infection was found only in the SRV group. The reason for this decrease can be explained by the biofilm complex in the SRV vaccine. However, despite the presence of the same antigenic complex in the BV_2, there was no statistically significant decrease in the BV_2 group compared to the control group. This supports that the Startvac® vaccine, which is not used at the standard dose, may not provide effective protection against *S. aureus* infection. Additionally, it can be stated that species-specific vaccines play a more effective role in the control of *S. aureus* mastitis. Mastitis vaccines used in this study decreased the NAS mastitis rate, but no statistically significant difference was observed. It may be related to the species heterogeneity of NAS [4,6]. Another reason may be related to the biofilm production potential of the NAS isolated from the herd [19,39]. However, the effect of microorganisms on biofilm production was not determined in this study. Examining the biofilm potentials of the isolated pathogens in the study could have provided more comprehensive and definitive results. Biofilm production can affect the effects of microorganisms causing mastitis and their susceptibility to vaccines. Therefore, evaluating the biofilm potentials in future studies could provide more information about the control of mastitis and the effectiveness of vaccines.

A significant decrease in *S. aureus* clinical mastitis rates was reported as a result of vaccination [40]. In another study, heifers were administered a vaccine prepared from 2-8 and Smith strains, which were isolated from clinical mastitis cases [29]. The vaccine group did not encounter any clinical mastitis cases. However, the subclinical mastitis rates were 8% and 16% in the vaccine and control groups, respectively. In contrast to the aforementioned studies, some researchers administered a commercial inactivated polyvalent vaccine (containing *S. aureus* TC 5, TC 8 strains, *E. coli* J5 strain, *S. agalactiae*, *S. uberis*, *S. dysgalactiae*, *S. pyogenes*, *P. aeruginosa*, and *T. pyogenes*) in cattle [21]. The study results revealed a clinical mastitis rate of 18.7% in the control group and 26.1% in the vaccine group. Consequently, it was concluded that the vaccine did not have a significant impact in reducing the incidence of clinical mastitis. In this study, it was found that the clinical mastitis incidence decreased in all vaccine groups compared to the control group, and a statistically significant difference was found between the BV_2 and SRV groups compared to the control group ($P < 0.01$). The reason for this difference is that leads to clinical mastitis is mostly gram-negative agents. Contrary to this situation, although clinical mastitis rates decreased in the BV_1 group compared to the control group, it is estimated that this rate increased in clinical mastitis due to gram-negative agents in the BV_1 group compared to other vaccine groups. Furthermore, the decrease in subclinical infections in these groups may have prevented the escalation of the infection.

Some researchers reported a moderate reduction in the rates of new infections caused by *S. aureus* and NAS with the Startvac® vaccine, while others found that the Lysigin® vaccine did not have a significant effect on the rates of new intramammary infections caused by *S. aureus* and NAS ($P \geq 0.05$) [27,36]. Consistent with these studies, the use of vaccines in this study resulted in a reduction in new infections caused by *S. aureus* and NAS. However, there was no statistically significant difference in the reduction of new infection rates between the BV_1 and BV_2. The only

statistically significant difference was observed in the SRV group, which is specific to the species.

In a different study, Lysigin® vaccine group had a spontaneous treatment rate of 1.28 (spontaneous treatment/goat), whereas the control group had a rate of 0.6 (spontaneous treatment/goat) [20]. In this study, a similar increase in spontaneous treatment rate was observed. However, in contrast to the previous study, no statistical difference was found between the groups in this study. The spontaneous treatment rates were very similar between the groups. The reason for the high rate of spontaneous treatment in this study can be explained by the fact that the animals were young and in their 1st lactation. When evaluated in terms of spontaneous treatment, it can be observed that the SRV group has a higher rate of spontaneous treatment for NAS mastitis. This can be explained by the more effective protection and immune response provided by the adjuvant present in the full dose of the vaccine. This is because during the administration of other vaccines, the vaccine dose was reduced to almost half.

There are many studies reporting the effect of mastitis vaccines on SCC [3,20,26]. The Vimco® vaccine was utilized by researchers in Lacaune and Manchega sheep, and it was reported that SCC significantly decreased in both breeds following vaccination. The mean SCC decreased from 563.049 cells/mL to 361.970 cells/mL in Lacaune sheep. It was found 1.060.938 cells/mL and 686.811 cells/mL in Manchega sheep control and vaccine groups, respectively [11]. These results are proportionally similar to the SRV group in this study. However, there was a difference between species in terms of SCC. This situation can be explained primarily by species diversity and apocrine lactation physiology [2,30]. In a study conducted, the Lysigin vaccine was used in Saanen goats, and the SCC was determined as 1.274 x 10^3 cells/mL and 1.529 x 10^3 cells/mL in the vaccine and control groups, respectively ($P < 0.10$) [20]. In this study, in the BV_1 group in which the same vaccine was used, the mean values in the vaccine and control groups were found to be 877.9 ± 53.5 x 10^3 - 1012.1 ± 62.6 x 10^3, respectively. The reason for the low SCC in this study, as in the other study, can be attributed to the fact that the goats were in their first lactation period.

CONCLUSIONS

As a result, it was observed that different mastitis vaccines used in small ruminants under field conditions generally reduced the mastitis incidence and decreased the SCC. In addition, positive effects on chronic infection and recovery rates were detected in the vaccines used. Therefore, it was thought that bovine-licensed vaccines used in small ruminants could be an alternative to small-ruminant-licensed vaccines. However, it is evident that the use of species-specific vaccines significantly reduced the rates of total *S. aureus* mastitis, subclinical NAS mastitis, and new NAS infections compared to other vaccines. Furthermore, the species-specific vaccine significantly increased the rate of spontaneous treatment for *S. aureus* mastitis. Additionally, this vaccine demonstrated the lowest somatic cell count values. Considering that *Staphylococcus* spp. is the most common causative agent in goats and the biofilm virulence of this microorganism, the presence of biofilm antigen in the vaccine to be administered can effectively reduce the rate of mastitis. However, it was determined that none of the mastitis vaccines eliminated totally the udder infection. Therefore, in addition to vaccination, other udder health management measures such as training of milkers, milking routine, maintenance of the milking system, and separation of infected animals from the herd should be included in the prevention of mastitis. It was thought that expanding the research and using more milk samples would increase the knowledge.

MANUFACTURERS

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***Ethical approval*.** This study was approved by the Experimental Animals Local Ethics Committee (MAKUHADY-EK/2016-28/181), Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Turkey. All procedures were carried out via the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 **Albenzio M., Taibi L., Muscio A. & Sevi A. 2002.** Prevalence and etiology of subclinical mastitis in intensively managed flocs and related changes in the yield and quality of ewe milk. *Small Ruminant Research*. 43(3): 219-226.

2 **Bergonier D., Cremoux R.D., Rupp R., Lagriffoul G & Berthelot X. 2003.** Mastitis of dairy small ruminants. *Veterinary Research*. 34(5): 689-716.

3 **Chang B.S., Moon J.S., Kang H.M., Kim Y.I., Lee H.K., Kim J.D., Lee B.S., Koo H.C. & Park Y.H. 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*. 26(17): 2081-2091.

4 **Contreras A., Corrales J.C., Sierra D. & Marco J. 1995.** Prevalence and etiology of non-clinical intramammary infection in Murciano-Granadina goats. *Small Ruminant Research*. 17(1): 71-78.

5 **Cucarella C., Tormo M.A., Ubeda C., Trotonda M.P., Monzon M., Peris C., Amorena B., Lasa I. & Penades J.R. 2004.** Role of biofilm-associated protein Bap in the pathogenesis of bovine *Staphylococcus aureus*. *Infection and Immunity*. 72(4): 2177-2185.

6 **Deinhofer M. & Pernthaner A. 1995.** *Staphylococcus* spp. as mastitis-related pathogens in goat milk. *Veterinary Microbiology*. 43(2-3): 161-166.

7 **Diaz J.R., Romero G., Muelas R., Alejandro M. & Peris C. 2012.** Effect of intramammary infection on milk electrical conductivity in Murciano-Granadina goats. *Journal of Dairy Science*. 95(2): 718-726.

8 **Dogruer G., Saribay M.K., Aslantas O., Kirecci E., Ergun Y., Ulku A. & Demir C. 2016.** The prevalance, etiology and antimicrobial susceptibility of the microorganisms in subclinical mastitis in goats. *Veterinary Sciences and Practices*. 11(2): 138-145.

9 **Dore S., Liciardi M., Amatiste S., Bergagna S., Bolzoni G., Caligiuri V., Cerrone A., Farina G., Montagna C.O., Saletti M.A., Scatassa M.L., Sotgiu G. & Cannas E.A. 2016.** Survey on small ruminant bacterial mastitis in Italy, 2013-2014. *Small Ruminant Research*. 141: 91-93.

10 **Felipe V., Morgante C.A., Somale P.S., Varroni F., Zingaretti M.L., Bachetti R.A., Correa S.G. & Porporatto C. 2007.** Evaluation of the biofilm forming ability and its associated genes in *Staphylococcus* species isolates from bovine mastitis in Argentinean. *Microbial Pathogenesis*. 104: 278-286.

11 **Fernandez J.A., García Carrero F., Delgado Rodríguez C. & Sanz Franco M.A 2018.** Efficacy of vaccination against biofilm-producing Staphylococci as a preventive measure against subclinical mastitis in Lacaune and Manchega sheep in Spain Rodríguez. In: *International Bovine Mastitis Congress* (Milano-Italy).

12 **Fthenakis G.C. 1994.** Prevalence and etiology of subclinical mastitis in ewes of southern Greece. *Small Ruminant Research*. 13(3): 293-300.

13 **Gelasakis A.I., Angelidis A.S., Giannakou R., Filioussis G., Kalamaki M.S. & Arsenos G. 2016.** Bacterial subclinical mastitis and its effect on milk yield in low-input dairy goat herds. *Journal of Dairy Science*. 99(5): 1-11.

14 **Hogan J. & Smith K.L. 2003.** Coliform mastitis. *Veterinary Research*. 34: 507-519.

15 **Hristov K., Popova T., Pepovich R. & Nikolov B. 2016.** Characterization of microbial causative agents of subclinical mastitis in goats in Bulgaria. *International Journal of Current Microbiology and Applied Sciences*. 5(8): 316-323.

16 **International Dairy Federation. 1981.** *Laboratory Methods for Use in Mastitis Work*. Brussels, Belgium. (Document no: 132).

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

18 **Ilhan Z., Tasal I., Sagcan S. & Solmaz H. 2011.** Isolation of aerobic bacteria from goat milk with subclinical mastitis. *Van Veterinary Journal*. 22(2): 89-91.

19 **Kahinda C.T.M. 2022.** Mastitis in Dairy Cattle, Sheep and Goats. In: Dego O.K. (Ed). *Mastitis in Small Ruminants*. London: IntechOpen, pp.87-114.

20 **Kautz F.M., Nickerson S.C. & Ely L.O. 2014.** Use of a staphylococcal vaccine to reduce prevalence of mastitis and lower somatic cell counts in a registered Saanen dairy goat herd. *Research in Veterinary Science*. 97(1): 18-19.

21 **Keskin A., Seyrek-Intas K., Tek H.B., Tuna B., Yılmazbas G., Ozakin C. & Ertas S. 2007.** Efficiency of polyvalent mastitis vaccine in lactating dairy cows. *Journal of Biological and Environmental Sciences*. 1(2): 87-92.

22 **Leitner G., Yadlin N., Lubashevsy E., Ezra E., Glickman A., Chaffer M., Winkler M., Sarana A. & Trainin Z. 2003.** Development of a *Staphylococcus aureus* vaccine against mastitis in dairy cows. II. field trial. *Veterinary Immunology and Immunopathology*. 93(3-4): 153-158.

23 **Leitner G., Lubashevsky E., Glickman A., Winkler M., Sarana A. & Trainin Z. 2003.** Development of a *Staphylococcus aureus* vaccine against mastitis in dairy cows I. Challenge trials. *Veterinary Immunology and Immunopathology*. 93(1-2): 31-38.

24 **Mahlangu P., Maina N. & Kagira J. 2018.** Prevalence, risk factors, and antibiogram of bacteria isolated from milk of goats with subclinical mastitis in Thika East Subcounty, Kenya. *Journal of Veterinary Medicine*. 2018: ID 3801479 DOI: 10.1155/2018/3801479

25 **Marogna G., Pilo C., Vidili A., Tola S., Schianchi G. & Leori S.G. 2012.** Comparison of clinical findings, microbiological result, and farming parameters in goats herds affected by recurrent infectious mastitis. *Small Ruminant Research*. 102(1): 74-83.

26 **Mella A., Ulloa F., Valdes I., Olivares N., Ceballos A. & Kruze J. 2017.** Evaluation of a new vaccine against *Staphylococcus aureus* mastitis in dairy herds of southern Chile I challenge trial. *Australian Journal Veterinary Science*. 49(3): 149-160.

27 **Middleton J.R., Luby C.D. & Adams D.S. 2009.** Efficacy of vaccination against staphylococcal mastitis. A review and new data. *Veterinary Microbiology*. 134(1-2): 192-198.

28 **National Mastitis Council. 2017.** *Laboratory Handbook on Bovine Mastitis. National Mastitis Council Inc*. (New Prague, USA). 148p.

29 **Nordhaug M.L., Nesse L.L., Norcross M.L. & Gudding R. 1994.** A field trial with an experimental vaccine against Staphylococcus aureus mastitis in cattle. 1. Clinical parameters. *Journal of Dairy Science*. 77(5): 1267-1275.

30 **Paape M.J., Capuco A.V., Contreras A. & Marco J.C. 2001.** Milk somatic cells and lactation in small ruminants. *Journal of Dairy Science*. (84): 237-244.

31 **Pereira U.P., Oliveira D.G., Mesquita L.R., Costa G.M. & Pereira L.J. 2011.** Efficacy of *Staphylococcus aureus* vaccines for bovine mastitis. A systematic review. *Veterinary Microbiology*. 148(2-4): 117-124.

32 **Persson Y., Jarnberg A., Humblot P. & Nyman A. 2015.** Associations between *Staphylococcus aureus* intramammary infections and somatic cell counts in dairy goat herds. *Small Ruminant Research*. 133: 62-66.

33 **Pir Yagcı I. & Kaymaz M. 2006.** Detection of subclinical mastitis by clinical, microbiological and biochemical methods in sheep. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*. 53: 31-35.

34 **Rovai M., Caja G., Salama A.A.K., Jubert A., Lázaro B., Lázaro M. & Leitner G. 2014.** Identifying the major bacteria causing intramammary infections in individual milk samples of sheep and goats using traditional bacteria culturing and real-time polymerase chain reaction. *Journal of Dairy Science*. 97(9): 5393-5400.

35 **Ruiz P., Barragan I., Sesena S. & Palop M.L. 2016.** Is staphylococci population from milk of healthy goats safe? *International Journal of Food Microbiology*. 238: 146-152.

36 **Schukken Y.H., Bronzo V., Locatelli C., Pollera C., Rota N., Casula A., Testa F., Scaccabarozzi L., March R., Zalduendo D., Guix R. & Moroni P. 2014.** Efficacy of vaccination on *Staphylococcus aureus* and coagulase-negative staphylococci intramammary infection dynamics in 2 dairy herds. *Journal of Dairy Science*. 97(8): 5250-5264.

37 **Tenhagen B.A., Edinger D., Baumgartner B., Kalbe P, Kluender G. & Heuwieser W. 2001.** Efficacy of a herd-specific vaccine against *Staphylococcus aureus* to prevent post-partum mastitis in dairy heifers. *Journal of Veterinary* Medicine. 48(10): 601-607.

38 **Vasileiou N.G.C., Chatzopoulos D.C., Cripps P.J., Ioannidi K.S., Gougoulis D.A., Chouzouris T.M. & Fthenakis G.C. 2019.** Evaluation of efficacy of a biofilm-embedded bacteria-based vaccine against staphylococcal mastitis in sheep-A randomized, placebo controlled field study. *Journal of Dairy Science*. 102(10): 9328-9344.

39 **Vasileiou N.G.C., Chatzopoulos D.C., Gougoulis D.A., Sarrou S., Katsafadou A.I., Spyrou V., Mavrogianni V.S., Petinaki E. & Fthenakis G.C. 2018.** Slime-producing staphylococci as causal agents of subclinical mastitis in sheep. *Veterinary Microbiology*. 224: 93-99.

40 **Watson D.L., McColl M.L. & Davies H.I. 1996.** Field trial of a staphylococcal mastitis vaccine in dairy herds: clinical, subclinical and microbiological assessments. *Australian Veterinary Journal*. 74(6): 447-450.

41 **White E.C. & Hinckley L.S. 1999.** Prevalence of mastitis pathogens in goat milk. *Small Ruminant Research*. 33(2): 117-121.