

Vaccination as a Tool to Reduce Mastitis and Improve Milk Quality in Dairy Goats

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Abstract

The purpose of this investigation was to evaluate the efficacy of a staphylococcal bacterin (Lysigin[®]) in reducing the prevalence of staphylococcal mastitis and somatic cell counts (SCC) in a commercial dairy goat herd. Does were vaccinated (n = 15) or left as controls (n = 15) and the levels of mastitis and SCC were monitored at approximately 6-wk intervals over an 18-mo period. Prior to and after vaccination, *Staphylococcus caprae* (42.5%), *S. xylosus* (15.1%), and *S. simulans* (10.0%) were the predominant causes of intramammary infections (IMI) in the herd. The new infection rate was 1.64 IMI/doe among vaccinates, which was lower than but not different ($P < 0.12$) from controls (2.67 IMI/doe). The majority of new IMI across treatments were caused by *S. caprae* (31.7%) and *S. xylosus* (23.9%). The spontaneous cure rate of existing IMI after immunization was 1.28 cures/doe in vaccinates, which was higher than that observed in controls (0.6 cures/doe; $P < 0.043$); the majority of spontaneous cures occurred with *S. caprae* (44.4%) and *S. xylosus* (22.3%). Average SCC from milk samples of vaccinated does over the trial showed a tendency to be lower than that of nonvaccinated controls (1274 x 10³/ml vs. 1529 x 10³/ml, respectively) ($P < 0.10$). In addition, bulk tank SCC averaged for the 5 pre-vaccination sampling dates was 1293 x 10³/ml, and for the 14 post-vaccination dates, SCC averaged 1052 x 10³/ml. The freezing of milk samples had no deleterious effect on determining SCC. Results support the continued study of mastitis vaccines for use in managing staphylococcal mastitis and SCC in dairy goats.

Introduction

Prevalence of mastitis in small ruminants such as goats and sheep ranges between 5 and 30%, with *Staphylococcus* spp., otherwise known as the coagulase-negative staphylococci (CNS), identified as the most frequent isolates (Contreras et al., 2007). The CNS are less pathogenic than *Staphylococcus aureus*, but produce persistent subclinical mastitis with markedly elevated somatic cell counts (SCC), which may lead to clinical symptoms (Deinhofer and Pernthaner, 1995; Contreras et al., 1997.)

Prevention is the key to controlling staphylococcal mastitis in dairy goats, as once this disease is established, chronic inflammation of mammary tissues and elevated SCC will likely ensue, resulting in reduced milk yield (Leitner et al., 2004a, b). The result is a reduction in farm income and potential loss of the infected udder half, which may ultimately lead to the loss of the animal either through culling or death, especially if gangrenous mastitis occurs. Natural transmission of this contagious disease throughout the herd by the milking machine and milker's hands underlies the need for prevention.

Vaccination as a preventative measure against *S. aureus* and the CNS in small ruminants has been attempted with variable results. Derbyshire (1961) vaccinated goats with live *S. aureus* and showed some immunity to a massive intramammary infusion of the same bacteria, but a subsequent study showed that after intramammary immunization, incidence of new

intramammary infections (IMI) was not reduced (Derbyshire and Smith, 1969). Similarly, vaccinating ewes against staphylococcal mastitis demonstrated no effect on preventing subclinical IMI (Marco, 1994). The staphylococcal vaccine, Lysigin[®] (Boehringer Ingelheim, Vetmedica, Inc., St. Joseph, MO), when administered to adult cows was shown to improve spontaneous cure rates of existing IMI but had no effect on preventing new cases of mastitis (Pankey et al., 1985). However, this vaccine was successful in preventing new *S. aureus* and CNS infections in dairy heifers, thereby reducing SCC and increasing production in the first lactation (Nickerson et al., 1999, 2009).

The purpose of this study was to determine the efficacy of Lysigin[®] over an 18-mo period in reducing the development of new cases of staphylococcal mastitis and lowering the herd SCC in a commercial Grade A Saanen dairy goat herd in Georgia (USA) that was in jeopardy of losing its milk market due to elevated herd SCC.

Materials and Methods

This investigation included 30 Saanen does at various stages of lactation in a commercial milking goat herd. Does were milked twice daily in a parlor using DeLaval milking equipment. Strict milking hygiene was followed including pre- and postmilking sanitization of teats using a chlorine-based germicide. Use of animals was approved by the University of Georgia (UGA) Institutional Animal Care and Use Committee. All procedures were carried out according to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Goats were divided into two groups: 1) vaccinates (n = 15) and 2) nonvaccinated controls (n = 15), and balanced by udder infection status, days in milk, average daily milk yield, and SCC. The vaccinated does were inoculated with a 3-ml dose of Lysigin[®] intramuscularly in the right semimembranosus muscle of the rear leg following the prevaccination milk sampling. Vaccination was repeated at 6-mo intervals thereafter, with a booster given 2 wk after the initial vaccination per label instructions. Injection sites were alternated between right and left sides and were also monitored for any adverse reactions. Due to normal herd culling, 14 does remained in the vaccinated group and 15 in the control group by the end of the trial.

Milk samples were taken aseptically from each udder half of all does in the herd 3 times at monthly intervals prior to vaccination and at approximately 6-wk intervals throughout an 18-mo period. Sampling procedures included sanitizing each teat end with a 70% isopropyl alcohol swab before machine milking. After sanitation, milk samples were collected into sterile disposable vials, placed in a cooler, and transported to the UGA Mastitis Lab. Aliquots of 0.01 ml of each sample were plated via inoculating loops onto Tryptic Soy Agar blood plates and incubated for 48 h at 37^oC. All milk samples were classified as visibly normal, and halves from which bacteria were isolated were classified as subclinically infected as no clinical symptoms were observed in study animals during the trial.

After presumptive identification based on colony morphology and hemolytic patterns, bacteria were further identified. Staphylococci were differentiated from streptococci by means of the catalase test. Conclusive identification of *Streptococcus* spp. was then verified by means of the

API Strep Test (bioMerieux, Inc., Marcy l'Etoile, France). Staphylococci were differentiated as coagulase positive or negative by conducting the coagulase test as follows: A loopful of colonial growth from a pure culture was placed in 0.50 ml of Remel Coagulase Plasma (Lenexa, KS, USA) and incubated at 37°C for a period of up to 24 h. Coagulation within the 24-h time frame indicated a coagulase-positive staphylococcus, of which *S. aureus* is most common. Final verification of the bacterial species was performed using the API Staph test (bioMerieux, Inc.).

Over the post-vaccination trial period, the numbers of new IMI that were diagnosed in previously uninfected udder halves of vaccinates and controls were determined, and means, expressed on a per animal basis, were separated using SAS 9.3 Proc Glim for Windows (SAS, 2013). Additionally, over the post-vaccination trial period, the spontaneous cure rates of previously infected udder halves of vaccinates and controls were determined, and means, expressed on a per animal basis, were separated using SAS as above (SAS, 2013).

SCC were determined on all half udder milk samples collected before and during the trial using a DeLaval Cell Counter (DCC, DeLaval International AB, Tumba, Sweden). The DCC establishes the number of somatic cells per ml of milk by means of an interior digital camera, photographing cellular nuclei, which are stained with a DNA-specific fluorescent probe and subsequently amplified. The DCC was also used for determining the SCC of bulk tank milk samples that were collected before (5 samplings) and after (14 samplings) trial initiation. The DCC has been demonstrated to have a 95% correlation with the direct microscopic method of counting somatic cells in goat milk and is considered a reliable cell counting method (Berry and Broughan, 2007).

A total of 530 fresh milk samples, which were stored on ice for up to 24 h, were analyzed using the DCC and recorded. Subsequently, 489 of these samples were frozen at approximately -20 °C, and at a later date, they were thawed to room temperature and processed again through the DCC. Generally, samples were frozen from between 2 and 8 wk prior to recounting. The purpose of recounting was to determine whether SCC results from the processing of fresh milk samples could be accurately repeated after freezing of the same samples. This information was deemed important because, at times, it may be unfeasible to immediately analyze milk samples that have been freshly collected, thus requiring freezing of samples for storage over extended periods of time prior to counting. The SCC of fresh samples were compared with paired frozen samples (same date of collection) and means separated using SAS as above (SAS, 2013). After pairing, a total of 487 samples each of fresh and frozen samples were analyzed.

Results and Discussion

Overall prevalence of infected does (percentage of does with at least one udder half subclinically infected) across treatments during the 18-mo trial period was 68.1% (range 55 to 83%). This percentage of infected does is greater than the 5 to 30% previously reported in the literature as reviewed by Contreras et al. (2007) and underscores the focus of this trial in reducing the development of new IMI and lowering the herd SCC. Prevalence was slightly lower for vaccinates (64.0%, range 36 to 83%) compared with controls (71.7%, range 38 to 85%), but not different ($P < 0.05$). Likewise, examination of microbiological culture results showed that after herd immunization, 48.1% of udder half isolates from vaccinated does were culture positive for some type of mastitis pathogen, whereas in controls, 55.6% were culture positive (Table 1);

again, this difference was not significant ($P < 0.05$). Monitoring of injection sites demonstrated no adverse reactions to vaccination.

The frequencies of various bacterial isolates from udders of vaccinated and control does is presented in Table 2. Among vaccinated does, *S. caprae* made up 51.9% of positive isolates, followed by *S. xylosus* (12.6%), *Enterococcus faecium* (9.4%), *S. chromogenes* (7.9%), and various other staphylococci. Among nonvaccinated does, *S. caprae* made up 33.1% of positive isolates, followed by *S. xylosus* (17.6%), *S. simulans* (16.9%), *S. chromogenes* (8.1%), and various other staphylococci, streptococci, and gram-positive and gram-negative bacilli. Thus, the distribution of isolates in both treatment groups was similar except for more frequent isolations of *E. faecium* in vaccinates (9.4% vs. 0%) and more frequent isolations of *S. simulans* in controls (16.8% vs. 3.1%). Average frequencies across treatments showed that *S. caprae* (42.5%), *S. xylosus* (15.1%), and *S. simulans* (10.0%) were the predominant bacterial isolates (Table 2).

A comparison of the new IMI rate between treatments revealed a rate of 1.64 IMI/doe among vaccinates, which was lower but not significantly ($P < 0.122$) different from controls (2.67 IMI/doe) (Table 1). For example, over the trial, 23 new IMI occurred in 14 vaccinated does (12 new IMI in the right half and 11 in the left half), and 40 new IMI occurred in 15 control does (15 new IMI in the right half and 25 in the left half). Most of the new IMI in vaccinates were caused by *S. caprae* (52.2%) followed by *S. xylosus* (21.7%), *S. chromogenes* (13.1%), *S. epidermidis* (8.7%), and *Staphylococcus* spp. (4.3%). Most of the new IMI in controls were caused by *S. xylosus* (25.0%) followed by *S. caprae* (20.0%), *S. simulans* (15.0%), *S. capitis* (10.0%), *S. hyicus* (7.5%), *S. epidermidis* and *S. sciuri* both at 5.0%, and gram-positive bacillus, *S. aureus*, *S. chromogenes*, *S. lentus*, and *Staphylococcus* spp. each at 2.5%. Overall, the majority (55.6%) of new IMI were caused by *S. caprae* (31.7%) and *S. xylosus* (23.9%).

An evaluation of the spontaneous cure rate, or the ability of an infected udder half to resolve or cure naturally without antibiotic intervention revealed a cure rate of 1.28 cures/doe among vaccinates, which was significantly higher ($P < 0.043$) than the rate of 0.6 cures/doe among controls (Table 1). For example, over the trial, 18 infected halves of 14 does cured spontaneously among vaccinated animals (1.28/doe), and 9 infected halves of 15 does cured spontaneously among unvaccinated controls (0.6/doe). Most of the cures in vaccinates occurred with *S. caprae* (50%) followed by *S. xylosus* (22.2%), with *S. aureus*, *S. chromogenes*, *S. lentus*, *Staphylococcus* spp., and *E. faecium* each at 5.6% of cured halves. Most of the cures in controls occurred with *S. caprae* (33.3%) followed by *S. xylosus* (22.2%), with *S. chromogenes*, *S. hyicus*, *S. warneri*, and *Str. agalactiae* each at 11.1% of cured halves. Overall, the majority (66.7%) of cures occurred with *S. caprae* (44.4%) and *S. xylosus* (22.3%). This increase in spontaneous cure rate of staphylococcal IMI among vaccinated vs. control does was unexpected. However, a previous trial also demonstrated an increase in the spontaneous cure rate of *S. aureus* IMI over 3 lactations among cows vaccinated with Lysigin[®] compared with unvaccinated controls; SCC were also lower in vaccinates (Pankey et al., 1985). Likewise, Sears (1998) observed an increase in cure rates of *S. aureus* IMI and a decrease in SCC in response to antibiotic therapy among cows vaccinated with an autogenous *S. aureus* vaccine compared with unvaccinated controls.

The CNS or *Staphylococcus* spp. were the most prevalent cause of mastitis in this herd, with *S. caprae*, *S. xylosus*, and *S. simulans* representing the most prevalent microorganisms cultured

from milk samples across vaccinated and control groups. Likewise, the CNS were found to be the predominant isolates in goat herd studies by White and Hinckley (1999), McDougal et al. (2002), and Contreras et al. (1995). In the present trial, the predominant CNS species that caused IMI were *S. caprae*, *S. xylosum*, and *S. simulans*. Prevalences for *S. xylosum* and *S. simulans* in the present trial were similar to those observed by others based on a review of the literature by Contreras et al. (2007); however, the prevalence of *S. caprae* in the present trial was greater than what had been observed previously.

The average SCC of all samples collected before and after the trial regardless of treatment or infection status for fresh and frozen samples combined was $1403 \times 10^3/\text{ml}$. The SCC of all fresh samples (right and left sides combined) was not different from frozen samples ($1390 \times 10^3/\text{ml}$ vs. $1401 \times 10^3/\text{ml}$; $P < 0.733$) (Table 3). Similarly, there were no differences between fresh and frozen samples for right and left halves. Thus, the DCC was equally effective in enumerating somatic cells in goat milk samples that had been frozen for up to 2 mo as it was in enumerating cells in fresh milk samples.

Average SCC of vaccinated does for all fresh and frozen samples combined ($1274 \times 10^3/\text{ml}$) showed a tendency to be lower ($P < 0.10$) than that of control does ($1529 \times 10^3/\text{ml}$) (Table 4). A comparison of SCC among fresh and frozen samples taken from right udder halves for vaccinated and control does revealed that SCC were numerically higher in control does but not significantly different. However, among fresh and frozen samples taken from left udder halves, SCC were significantly elevated in control does compared with vaccinated does in fresh ($1615 \times 10^3/\text{ml}$ vs. $1155 \times 10^3/\text{ml}$; $P < 0.016$) as well as in frozen samples ($1605 \times 10^3/\text{ml}$ vs. $1170 \times 10^3/\text{ml}$; $P < 0.014$). The reason for the higher SCC in left udder halves in this study is unknown because frequencies of positive isolates across treatments in right and left halves were similar (51.5% and 52.5%, respectively). However, among control does, the frequency of positive isolates among left udder halves was slightly higher than right halves (59 vs. 53%).

The average SCC of $1403 \times 10^3/\text{ml}$ across all milk samples in the present study was similar to an earlier study of a herd of 15 lactating does, in which an overall mean SCC of $1200 \times 10^3/\text{ml}$ was found among samples taken monthly over an 8-mo period (Zeng and Escobar, 1995). Likewise, Hinckley (1990) reported that 56% of milking does produced milk with SCC in the range of $1000 \times 10^3/\text{ml}$ to $2000 \times 10^3/\text{ml}$, and Droke et al. (1993) reported an average of $1300 \times 10^3/\text{ml}$ for bulk tank goat milk. Thus, the average SCC reported herein for goat milk regardless of infection status is similar to previous reports.

In the present trial, average SCC for uninfected halves was $1001 \times 10^3/\text{ml}$ and average SCC for infected halves was $1805 \times 10^3/\text{ml}$. The value for uninfected halves ($1001 \times 10^3/\text{ml}$) is higher than that observed in does by Leitner et al. (2004b) for uninfected halves ($417 \times 10^3/\text{ml}$), but the value for infected halves ($1805 \times 10^3/\text{ml}$) was similar to halves experimentally infected with CNS ($1,750 \times 10^3/\text{ml}$) that was observed by Leitner et al. (2004b). Values for both uninfected and infected halves observed in the present study are in line with those summarized in a review by Ruegg (2011) that demonstrated a SCC range of $270 \times 10^3/\text{ml}$ to $2000 \times 10^3/\text{ml}$ for uninfected halves and $650 \times 10^3/\text{ml}$ to $4200 \times 10^3/\text{ml}$ for infected halves.

The average SCC for each bacterial species isolated is in Table 5. Among the CNS, the highest SCC were associated with infections caused by *S. simulans* (3253 x10³/ml) followed by *S. sciuri* (3109 x10³/ml), and *Staphylococcus* spp. (2286 x10³/ml). Overall, CNS SCC ranged between 1067 x10³/ml and 3253 x10³/ml. Within the vaccinated group, SCC were numerically lower at the end of the vaccine trial than prior to vaccination but differences were not different due to the large variation in counts; pre-vaccination SCC based on 3 sampling dates averaged 1534 x 10³/ml and post-vaccination SCC based on 11 sampling dates averaged 1203 x 10³/ml. Likewise, within the nonvaccinated control group, SCC averaged 1908 x 10³/ml for the prevaccination sampling dates and 1402 x 10³/ml for post-vaccination sampling dates but differences were not different due to the large variability.

Bulk tank SCC decreased over the 18-mo period (Figure 1). Bulk tank SCC averaged 1293 x 10³/ml for the 5 prevaccination sampling dates, and for the 14 post vaccination dates, SCC averaged 1052 x 10³/ml, which is a decrease of 241,000 cells/ml. The prevaccination bulk tank SCC (1293 x 10³/ml) is very similar to the average of 1300x10³/ml reported by Droke et al. (1993) for bulk tank goat milk.

Conclusions

Vaccination resulted in nonsignificant decrease in the new infection rate among the immunized goats and a significant increase in the spontaneous cure rate compared with unvaccinated controls. Likewise, SCC were reduced in vaccinated animals compared with controls (1267 x 10³/ml vs. 1525 x 10³/ml). The legal SCC limit for herd milk in dairy goats is 1500 x 10³/ml; thus even a small reduction in herd SCC (e.g. a reduction to 1267 x 10³/ml, as in this case for vaccinates) is sufficient to allow the sale of goat milk for human consumption and maintain the producer within the allowable SCC limit, supporting the continued evaluation of immunization for mastitis control in goats. The DCC was equally effective in enumerating somatic cells in goat milk samples that had been frozen for up to 2 mo as it was in enumerating cells in fresh milk samples; thus, this somatic cell counting device can be used to accurately determine the SCC in milk samples that need to be frozen prior to processing.

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Table 1. Frequencies of positive cultures, new infection rate, and spontaneous cure rate among vaccinated and unvaccinated does in commercial milking herd.

	Vaccinated	Control
Frequency of positive isolates ¹	48.1%	55.6%
New infections ²	1.64	2.67*
Spontaneous cures ³	1.28	0.60**

¹Percentage of milk samples from which bacteria were isolated over the 15-month trial.

²Number of new infections diagnosed in previously uninfected udder halves on a per animal basis over the length of the trial.

³Spontaneous cures of previously infected udder halves on a per animal basis over the length of the trial.

* $P < 0.122$.

** $P < 0.043$.

Table 2. Frequencies (%) of bacterial isolates among infected halves of vaccinated and control does.

Isolate	Vaccinated	Control	Avg
<i>Enterococcus faecium</i>	7.9	0	3.9
Gram-negative bacillus	0	2.0	1.0
Gram-positive bacillus	0	0.7	0.4
<i>Staphylococcus aureus</i>	1.6	3.4	2.5
<i>Staphylococcus capitis</i>	1.6	4.6	3.1
<i>Staphylococcus caprae</i>	51.9	33.1	42.5
<i>Staphylococcus chromogenes</i>	7.9	8.1	8.0
<i>Staphylococcus epidermidis</i>	5.6	2.7	4.2
<i>Staphylococcus hyicus</i>	0.8	2.7	1.8
<i>Staphylococcus lentus</i>	2.4	1.4	1.9
<i>Staphylococcus sciuri</i>	0	1.4	0.7
<i>Staphylococcus simulans</i>	3.1	16.8	10.0
<i>Staphylococcus</i> spp.	3.1	1.4	2.3
<i>Staphylococcus warneri</i>	0	2.7	1.4
<i>Staphylococcus xylosum</i>	13.4	17.6	15.5
<i>Streptococcus agalactiae</i>	0	1.4	0.7
<i>Streptococcus</i> spp.	0.8	0	0.4

Table 3. SCC differences between fresh and frozen milk samples from udder halves.

Variable	n	Fresh	Frozen	P value
Right half	244	1395 x 10 ³ /ml	1416 x 10 ³ /ml	0.525
Left half	243	1385 x 10 ³ /ml	1387 x 10 ³ /ml	0.907
Both halves	487	1390 x 10 ³ /ml	1401 x 10 ³ /ml	0.733

Table 4. SCC differences between vaccinated and control does for fresh and frozen milk samples.

Variable (SCC sample)	n	SCC Vaccinated	n	SCC Control	SE	P value
Right half fresh sample	132	1376 x 10 ³ /ml	133	1414 x 10 ³ /ml	143.3	0.850
Right half frozen sample	122	1367 x 10 ³ /ml	123	1464 x 10 ³ /ml	129.3	0.594
Left half fresh sample	132	1155 x 10 ³ /ml	132	1615 x 10 ³ /ml	134.3	0.016
Left half frozen sample	122	1170 x 10 ³ /ml	122	1605 x 10 ³ /ml	123.8	0.014
All SCC	508	1274 x 10 ³ /ml	510	1529 x 10 ³ /ml	108.7	0.100

Table 5. Somatic cell counts (SCC) associated with the various bacterial isolates from milk samples.

Isolate	SCC x 10 ³
Uninfected	1001
<i>Enterococcus faecium</i>	4520
Gram-negative bacillus	2503
Gram-positive bacillus	5778
<i>Staphylococcus aureus</i>	2019
<i>Staphylococcus capitis</i>	1890
<i>Staphylococcus caprae</i>	1526
<i>Staphylococcus chromogenes</i>	1067
<i>Staphylococcus epidermidis</i>	2112
<i>Staphylococcus hyicus</i>	1137
<i>Staphylococcus lentus</i>	1327
<i>Staphylococcus sciuri</i>	3109
<i>Staphylococcus simulans</i>	3253
<i>Staphylococcus</i> spp.	2286
<i>Staphylococcus warneri</i>	1430
<i>Staphylococcus xylosus</i>	1529
<i>Streptococcus agalactiae</i>	127
<i>Streptococcus</i> spp.	3026

Figure 1. Bulk tank SCC averages by month (1000/ml).

