Use of a *Staphylococcus aureus* vaccine to reduce prevalence of staphylococcal mastitis and lower somatic cell counts in a registered Saanen dairy goat herd

J. D. Williams, F. M. Kautz, L. O. Ely, and S. C. Nickerson

Abstract

The purpose of this investigation was to evaluate the efficacy of a commercial bacterin (Lysigin®) in reducing the prevalence of staphylococcal intramammary infections (IMI) and lowering somatic cell counts (SCC) in a commercial dairy goat herd. Does were sampled over a 4-month period prior to vaccination to establish a baseline for the prevalence of mastitis, SCC, and bulk tank bacteria count. All does were vaccinated, and the levels of mastitis and SCC were monitored at 4-week intervals over a 6-month period. The average IMI prevalence over the 4 months prior to vaccination was 77.0%, and *Staphylococcus caprae, S. xylosus, S. simulans*, and *S. capitis* were the predominant causes of IMI. Following vaccination, culture results revealed that although the distribution of isolates had not changed, the average prevalence of infection decreased to 63.9%. Likewise, the average number of mastitic udder halves per doe decreased from 1.2 prior to vaccination to 0.91 postvaccination. The average bulk tank SCC decreased from 1,118,625/ml during the prevaccination period to 886,600/ml during the postvaccination period, and the average bulk tank bacteria count decreased from 14,900 to 6,200/ml. Data analysis suggested that herd vaccination led to a short-term reduction in the overall prevalence of mastitis, which was supported by the reduction in the bulk tank SCC and bacteria count. Results support the continued study of vaccination for managing staphylococcal mastitis and SCC in dairy goats in lieu of antibiotics.

Introduction

The prevalence of mastitis in small ruminants such as goats and sheep ranges between 5 and 30%, with *Staphylococcus* species or the coagulase-negative staphylococci (CNS) being the most frequent types of IMI (Contreras et al., 2007). The CNS are less pathogenic than *Staphylococcus aureus* in that they cause fewer clinical cases and are less severe; however, CNS IMI are associated with elevated SCC and bacteria counts, which may lead to clinical signs of disease (Deinhofer and Pernthaner, 1995; Contreras et al., 1997).

In dairy goats, prevention is the key to controlling staphylococcal mastitis. After an infection is established, chronic inflammation of mammary tissues and elevated SCC will likely follow, with reduced milk quality and yield (Leitner et al., 2004a, b), resulting in economic losses to the dairy operation (Koop et al., 2012). Vaccination as a preventative measure against *S. aureus* and CNS IMI 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 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), which is labelled for controlling *S. aureus* IMI in adult cows, was shown to improve spontaneous cure rates of existing IMI and lower SCC but had no effect on preventing new cases of mastitis (Pankey et al., 1985). However, in another trial, this same vaccine (Lysigin®) was

successful in preventing new *S. aureus* and CNS infections in dairy heifers (Nickerson et al., 1999).

A previous study (Kautz et al., 2014) of the same goat herd investigated in the present trial demonstrated that vaccination with Lysigin® reduced the prevalence of mastitis and SCC over an 18-month period. In that trial, the average number of new IMI on a doe basis among vaccinates was 1.64 while the average number in controls was 2.67 (P = 0.12). In addition, the average number of spontaneous cures (established infections that resolved without antibiotic therapy) increased among vaccinates (1.28 cures/doe) compared with controls (0.6 cures/doe) (P = 0.043). The average SCC among vaccinated does was 1,274,000/ml, while the average SCC for the control does was 1,529,000/ml. It was concluded that vaccination was instrumental in reducing mastitis and lowering the SCC in that particular problem herd that might have otherwise been degraded due to unacceptable milk quality (Kautz et al., 2014).

In the spring of 2013, the goat herd referenced above was in jeopardy of exceeding the legal limit of 1,500,000/ml for bulk milk SCC, and the level of mastitis had reached a high of almost 80%. Thus, the objective of this investigation was to determine if the process of whole herd vaccination with a staphylococcal mastitis bacterin could result in a sufficient immune response to the CNS already established in this herd. Such a response may reduce the inflammatory effects of ongoing infections and/or result in spontaneous cures, e.g., functioning as a therapeutic vaccine, leading to a reduction in the prevalence of mastitis as well as SCC in this herd.

Materials and Methods

This project included 29 Saanen does at various stages of lactation in a commercial milking goat herd that was 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 (Effercept®). Use of animals was approved by the University of Georgia 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).

Milk samples were taken aseptically from each udder half of all does in the herd 4 times prior to vaccination over a 4-month period to establish a baseline for udder infection status and SCC as well as bulk tank SCC and bacteria counts using standard procedures (Nickerson et al., 1999). After presumptive identification of bacteria based on colony morphology and hemolytic patterns, bacteria were further identified. If a presumptive identification could not be made, a Gram stain of the isolate in question was prepared. Staphylococci were differentiated from streptococci by means of the catalase test, and final verification of the bacterial species (majority staphylococci) was performed using the API Staph test (bioMerieux, Inc. Marcy l'Etoile, France). SCC were determined on milk samples using a DeLaval Cell Counter (DCC, DeLaval International AB, Tumba, Sweden). In addition to testing the SCC in udder halves of each goat, a bulk tank sample was taken on each sampling date and tested for SCC and bacteria count to establish the overall herd infection and milk quality status.

Examination of the prevaccination mastitis status and bulk milk quality indicated that this herd was in jeopardy of losing its milk market due to an elevated bulk tank SCC approaching the legal limit of 1,500,000/ml for Grade A goat milk. This increase in SCC was due to an elevation in overall infection rate with CNS, which ranged between 75% and 77.8% during the months of

August, September, and October, 2013. It was deemed necessary to vaccinate all lactating does in the manner as described below, and instead of comparing vaccinated does with unvaccinated controls, a comparison of the rate of infection and milk quality in the 4 months prior to vaccination with the rate of infection and milk quality after whole herd vaccination was made. This increased the odds of reducing the infection rate among the entire herd, thereby maintaining the bulk tank SCC below the legal limit and keeping the herd milk quality "in grade" to ensure production of a saleable product.

Thus, all 29 goats were inoculated with a 3-ml dose of Lysigin® administered using a 5-cc syringe equipped with an 18-gauge needle intramuscularly in the right semimembranosus muscle of the rear leg. Vaccination was repeated at 2-month intervals thereafter for 6 months, with a booster given 2 weeks after the initial vaccination. For the booster injections as well as those given at 2-month intervals, the same procedure was followed. Injection sites were alternated between right and left sides and monitored for swelling, induration, and abscess formation for 1 week after vaccine administration.

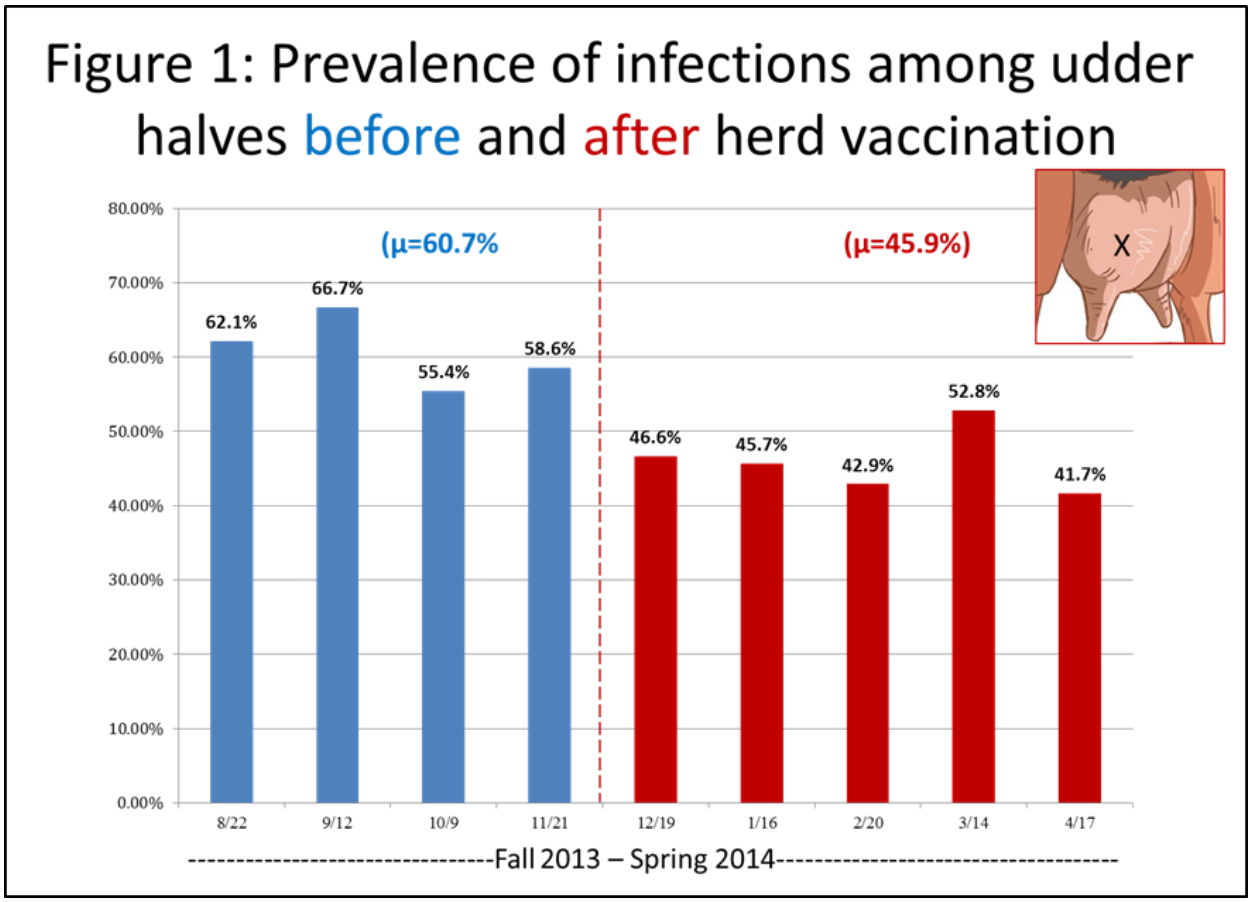
Beginning at 2 weeks after the vaccine booster was administered, milk samples from each udder half were taken to culture for presence of the mastitis pathogens as above (Nickerson et al., 1999), and SCC were recorded. Thereafter, milk samples were taken monthly and analyzed for the duration of the trial (6 months).

Prevalence of IMI among does (animals with at least 1 udder half infected) was determined as the percentage of infected does among the 29 animals on trial. Prevalence of IMI among udder halves was determined as the percentage of infected udder halves among the 58 udder halves of the 29 animals on trial. A new IMI developing over the trial was confirmed in an udder half that was uninfected for at least 2 consecutive samplings, which developed a new IMI that persisted for at least 2 consecutive samplings. Additionally, the ability of an infected udder half to resolve naturally without antibiotic intervention (spontaneous cure) was determined in udder halves pre- and postvaccination. A spontaneous cure was defined as an established IMI of at least 2 consecutive positive cultures of the same bacteria followed by at least 2 consecutive negative cultures.

The prevalence of IMI, as well as the SCC and bacteria counts during the months prior to vaccination, were compared to the prevalence after vaccination. The first vaccination was administered on October 24 and the booster was given 2 wk later, which was approximately 2 weeks prior to the first postvaccination sampling performed on November 21. The vaccination and boost most likely would have required at least a month to take effect, so the November 21 sampling was considered a prevaccination sampling, for a total of 4 prevaccination samplings, and these were compared to the postvaccination samplings performed December 19 through April 17. Data were analyzed and means separated using SAS (SAS, 2013).

Results and Discussion

Does did not show any swelling, induration, or abscess formation in response to the vaccinations. Results of the monthly herd samplings prior to vaccination demonstrated an unusually high prevalence of mastitis among udder halves (Figure 1) and does (Figure 2), which was reduced by herd vaccination. From August through November, the average prevaccination prevalence of infection among udder halves was 60.7%, and after vaccination, the average prevalence was 45.9% (Figure 1). Likewise, among does, the average prevaccination prevalence of infection was 77.0%, and after vaccination, the average prevalence was 63.9% (Figure 2).



Statistical comparisons of the prevalence of half udder infections (average number of udder halves with mastitis per doe) and animal infections (percentage of does with mastitis) by prevaccination and postvaccination period are in Table 1. The average number of udder halves with mastitis per doe decreased from 1.20 prevaccination to 0.91 postvaccination ($P < 0.008$), and the percentage of does with mastitis decreased from 0.770 to 0.639 ($P < 0.030$).

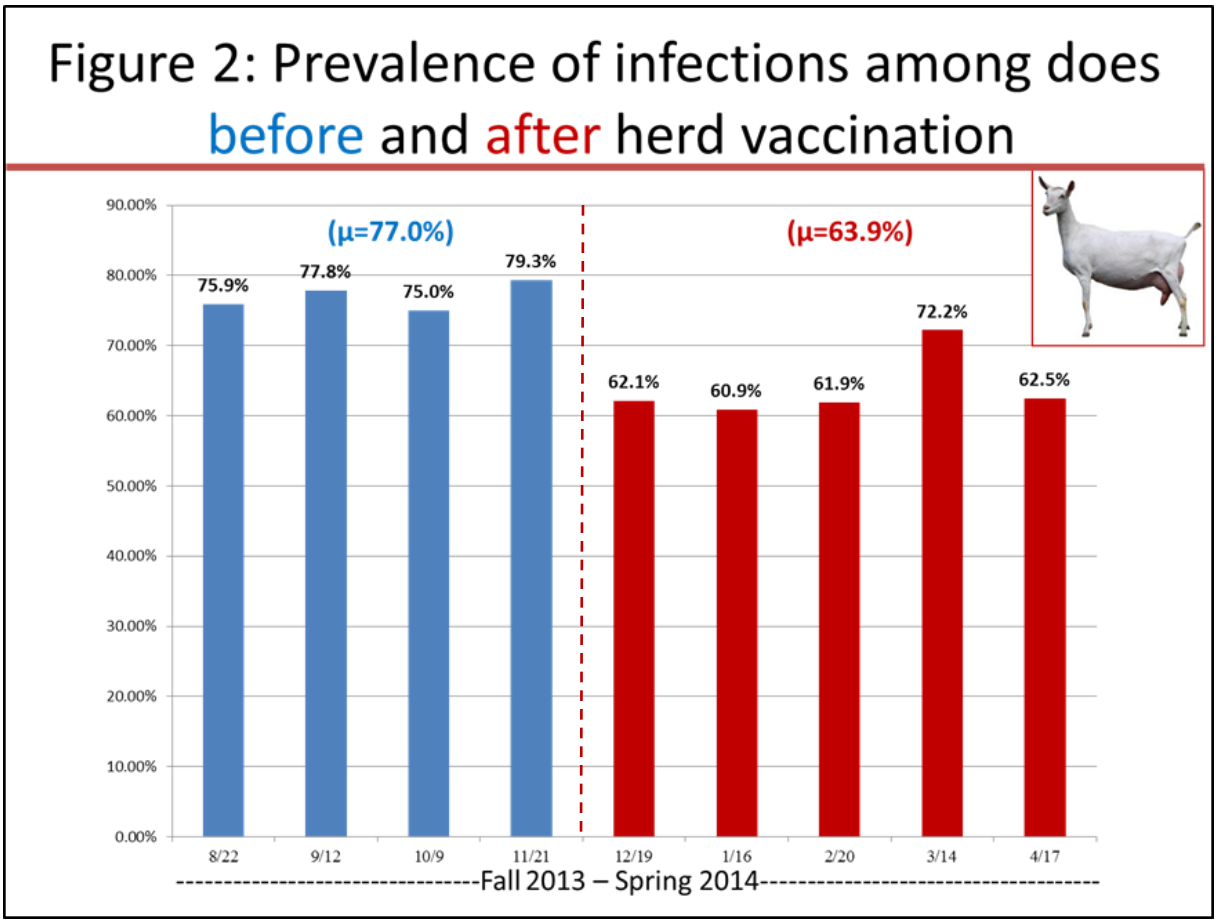
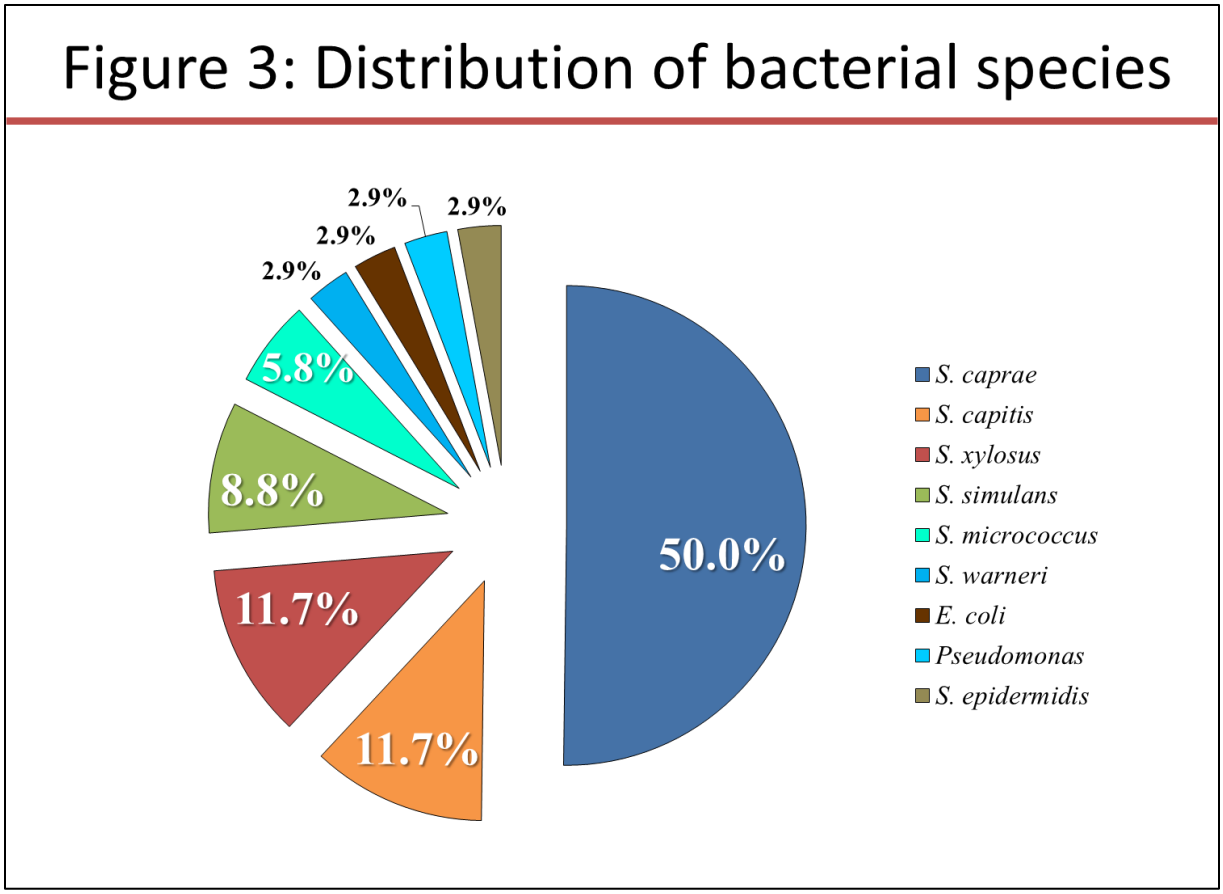


Table 1. Comparison of the prevalence of half udder infections*and animal infections** by prevaccination and postvaccination period.

	Prevaccination	Postvaccination	SE	*P* value
Half udder infections	1.20	0.910	0.07	0.008
Animal infections	0.77	0.639	0.04	0.030

*Average number of udder halves with mastitis per doe.
** Percentage of does with mastitis.

The frequency of infection with various bacterial isolates did not change over the trial; *S. caprae* (50.0%) was the most prevalent isolate followed by *S. capitis* (11.7%), *S. xylosus* (11.7%), and *S. simulans* (8.8%) (Figure 3).



An analysis of the development of new IMI indicated than only one new IMI was confirmed after vaccination, and it was a new IMI with *S. caprae* in one udder half (Table 2). On the other hand, spontaneous cures were confirmed in 8 udder halves after vaccination: 7 *S. caprae* IMI cured and 1 micrococcus IMI cured (Table 2). Kautz et al. (2014) also observed an increase in spontaneous cure rates among does immunized with a staphylococcal vaccine compared with unvaccinated controls, and in that study, the majority of cures occurred with *S. caprae* IMI as observed in the present trial.

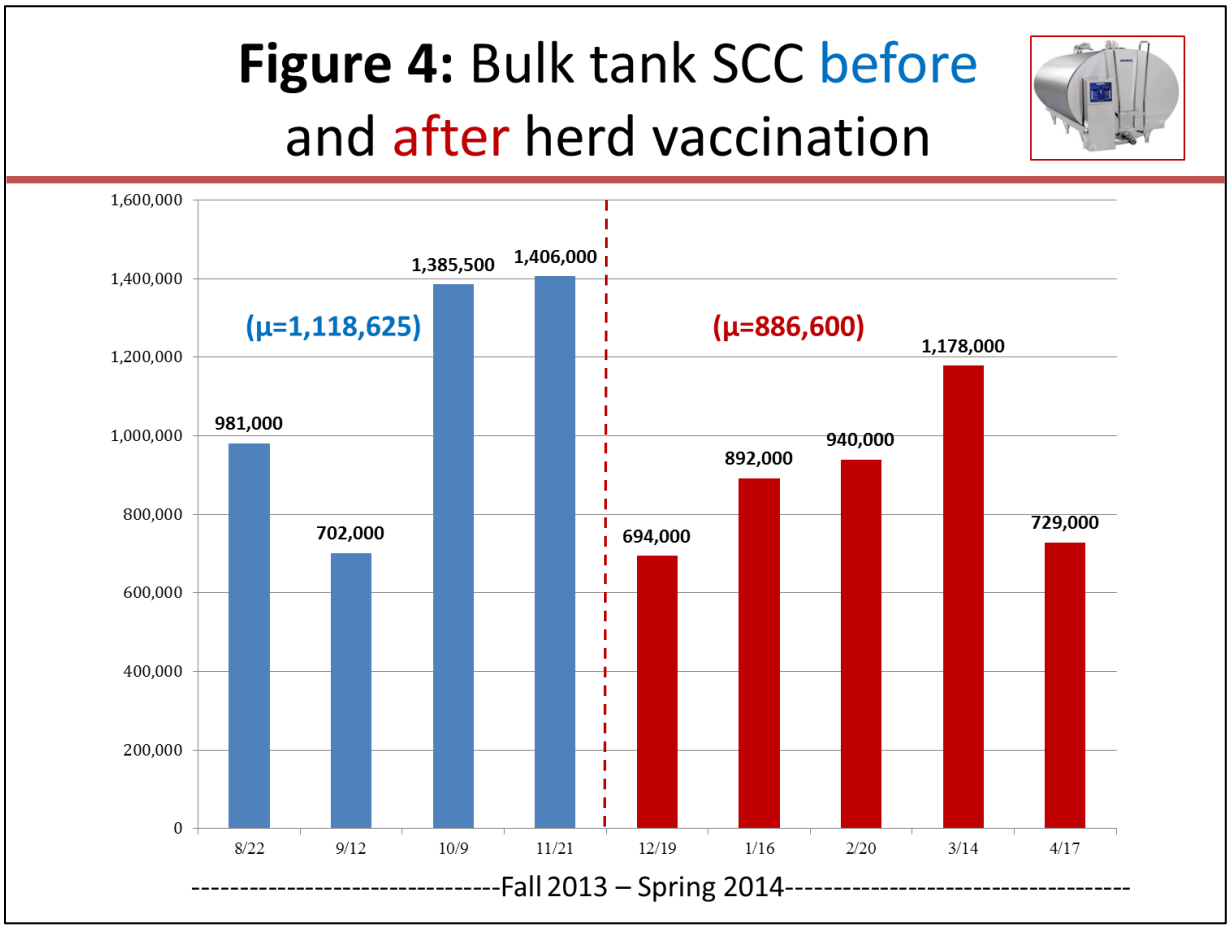
Table 2. Development of new IMI and spontaneous cure rate before and after vaccination.

	Prior to vaccination	After vaccination
New IMI (no.)	0	1[1]
Spontaneous cures (no.)	0	8[2]

[1] *S. caprae.*
[2] 7 *S. caprae*. 1 micrococcus.

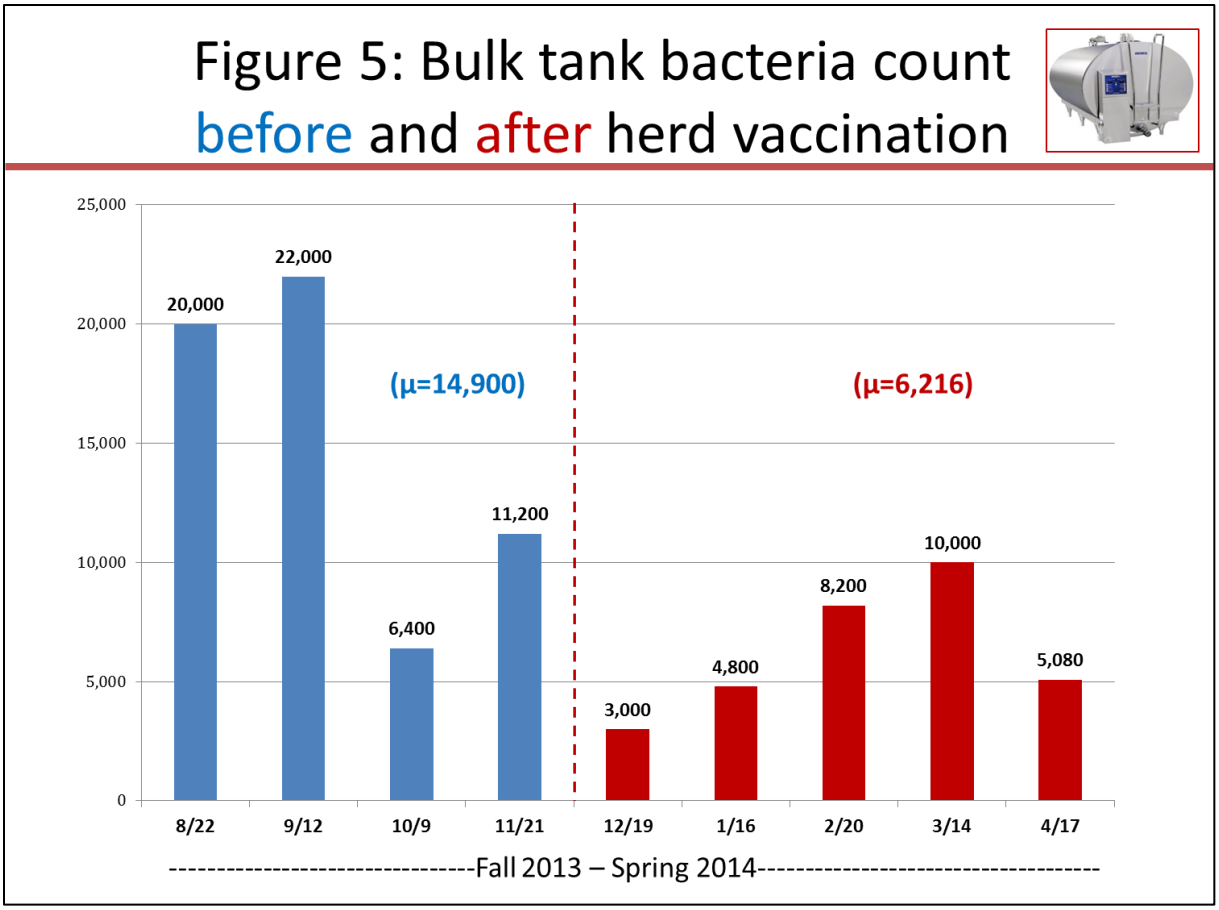
The average bulk tank SCC prior to vaccination was 1,118,625 with a low of 702,000 in September and a high of 1,406,000 in November (Figure 4). The average SCC after vaccination was 886,600 with a low of 694,000 in December and a high of 1,178,000 in March (Figure 4). The SCC in October at 1,385,500 prior to vaccination was quite elevated and dangerously close

to the 1,500,000 legal limit for salable milk but it decreased to 694,000 2 months later; however, the SCC slowly increased through the March sampling then decreased in April to 729,000.



In support of the lower SCC observed after vaccination, Kautz et al. (2014) observed that the average SCC of milk samples from vaccinated does showed a tendency to be lower than that of unvaccinated controls (1274 x 10^3 vs. 1529 x 10^3/ml). The average SCC across all milk samples in the present study (1100 x 10^3/ml) was similar to an earlier study of a herd of 15 lactating does, in which an overall mean SCC of 1200 x 10^3/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 x 10^3/ml to 2000 x 10^3/ml, and Droke et al. (1993) reported an average of 1300 x 10^3/ml for bulk tank goat milk. Thus, the average SCC reported herein for goat milk regardless of infection status is similar to previous reports. Interestingly, despite the overall reduction in bulk tank SCC, average individual doe SCC before and after vaccination did not differ (1,028,500 vs. 1,157,600/ml).

The bulk tank bacteria counts can be a measure of the quality of the milk being produced. Prior to vaccination, the bacteria counts recorded for each month from the bulk tank sample were elevated (>10,000/ml) and indicated an issue with overall milk quality (Figure 5). The average bacteria count from the bulk tank sample prior to vaccination was 14,900/ml, and after vaccination had been administered, the average count was 6,216/ml, demonstrating a general increase in milk quality and indicating a positive change in the overall herd health.



The CNS or *Staphylococcus* spp. were the most prevalent causes of mastitis in this herd, with *S. caprae*, *S. capitis*, *S. xylosus, and S. simulans* representing the most prevalent microorganisms cultured from milk samples both before and after vaccination. 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). Overall prevalence of infected does (percentage of does with at least one udder half subclinically infected) across the study (pre- and postvaccination) was 69.7% (range 60.9 to 79.3%). 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.

Conclusion

Vaccination lowered the overall prevalence of infection among does, and also lowered the SCC and bacteria count in the bulk tank. Although vaccination reduced mastitis and improved milk quality, the effects were limited and of short term, and further research is needed to optimize the use of a vaccination protocol to control mastitis in goats for a more long-term approach toward managing this disease.

References

Contreras, A., Corrales, J.C., Sierra, D., Marco, J. 1995. Prevalence and aetiology of nonclinical intramammary infection in Murciano–Granadina goats. Small Ruminant Research 17, 71–78.

Contreras, A., Paape, M.J., Di Carlo, A.L., Miller, R.B., Rainard, P. 1997. Evaluation of selected antibiotic residue screening tests for milk from individual goats. Journal of Dairy Science 80, 1113–1118.

Contreras, A., Sierra, D., Sánchez, A., Corrales, J.C., Marco, J.C., Paape, M.J., Gonzalo, C. 2007. Small Ruminant Research 68, 145–153.

Deinhofer, M., Pernthaner, A. 1995. *Staphylococcus* spp. as mastitis-related pathogens in goat milk. Veterinary Microbiology 43, 161–166.

Derbyshire, J.B. 1961. The immunization of goats against staphylococcus mastitis in the goat. Research in Veterinary Science 2, 112-121.

Derbyshire, J.B., Smith, G.S. 1969. Immunization against experimental staphylococcal mastitis in the goat by the intramammary infusion of cell-toxoid vaccine. Research in Veterinary Science 10, 559-564.

Droke, E.A., Paape, M.J., Di Carlo, A. L. 1993. Prevalence of high somatic cell counts in bulk tank goat milk. Journal of Dairy Science 76, 1035-1039.

Federation of Animal Sciences Societies, FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Federation of Animal Sciences Societies, Champaign, IL, USA.

Hinckley, L.S. 1990. Revision of the somatic cell count standard for goat milk. Dairy, Food and Environmental Sanitation 10, 548-549.

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. 97,18-19.

Koop, G., Oosterhuis, G., Nielen, M., Van Werven, T., Hogeveen, H. 2012. Estimating the costs of mastitis in goats using stochastic simulation modeling. Page 405 in Proceedings of the 13th International Symposium on Veterinary Epidemiology and Economics, Belgium, Netherlands.

Leitner, G., Chaffer, M., Shamay, A., Shapiro, F., Merin, U., Ezra, E., Saran, A., Silanikove, N. 2004a. Changes in milk composition as affected by subclinical mastitis in sheep. Journal of Dairy Science 87, 46–52.

Leitner, G., Merin, U., Silanikove, N. 2004b. Changes in milk composition as affected by subclinical mastitis in goats. Journal of Dairy Science 87, 1719–1726.

Marco, J.C. 1994. Mastitis in Latxa sheep breed, epidemiology, diagnosis and control. Doctoral Thesis. University of Zaragoza, 383 pp.

McDougall, S., Pankey, W., Delaney, C., Barlow, J., Murdough, P.A., Scruton, D. 2002. Prevalence and incidence of subclinical mastitis in goats and dairy ewes in Vermont, USA. Small Ruminant Research 46,115-121.

Nickerson, S.C., Owens, W. E., Tomita, G. M., Widel, P.W. 1999. Vaccinating dairy heifers with a Staphylococcus aureus bacterin reduces mastitis at calving. Large Animal Practice 20, 16-20.

Pankey, J.W., Boddie, N.T., Watts, J.L., Nickerson, S.C. 1985. Evaluation of protein A and a commercial bacterin as vaccines against *Staphylococcus aureus* mastitis by experimental challenge. Journal of Dairy Science 68,726-731.

SAS. 2013. Business and Analytical Software. SAS Institute, 100 SAS Campus Drive, Cary, NC.

White, E. C., Hinckley, L.S. 1999. Prevalence of mastitis pathogens in goat milk. Small Ruminant Research 33,117-121.

Zeng, S.S., Escobar, E.N. 1995. Effect of parity and milk production on somatic cell count, standard plate count and composition of goat milk. Small Ruminant Research 17, 269-274.