RESEARCH ARTICLE

The effects of commercial mastitis vaccine applied at different stages of lactation on the somatic cell count in individual and bulk tank milk in primiparous and multiparous cows in Turkey

Ayhan Baştan[1]*, Seçkin Salar[1], Doğukan Özen[2]

[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Ankara University, Dışkapı, Ankara, Turkey,
[2]Department of Biostatistics, Faculty of Veterinary Medicine, Ankara University, Dışkapı, Ankara, Turkey
Geliş:19.07.2017, Kabul: 26.09.2017
*abastan@ankara.edu.tr

Türkiye'de primipar ve multipar ineklerde laktasyonun farklı dönemlerinde uygulanan ticari mastitis aşının bireysel ve tank sütü somatik hücre sayısı üzerine etkisi

Öz

Amaç: Bu çalışmanın amacı, önerilenden farklı bir aşılama protokolü ile mastitis aşısı uygulamasının laktasyonun farklı primipar ve multipar ineklerde ve tank sütünde somatik hücre sayısı (SHS) üzerine etkisini araştırmaktı.

Gereç ve Yöntem: Çalışmada 97 baş sağlıklı Holştayn ırkı inek kullanıldı. Tüm ineklere 0. gün, 21. gün ve 111. gün olmak üzere 3 doz kas içi yolla inaktif mastitis aşısı enjekte edildi. İlk aşılamadan 30 gün önce ve 2. ile 3. aşılamadan sonraki 30. günlerde SHS analizi için bireysel ve süt toplama tankından süt örnekleri toplandı.

Bulgular: Aşılama öncesi, 2. ve 3. aşılama sonrası tank sütü SHS sırasıyla 467, 246, 371 x1000 hücre/ml olarak belirlendi. İkinci ve 3. aşılama sonrası ortalama süt SHS'de rakamsal bir düşüş şekillendiği belirlendi (p>0.05). Erken, orta veya geç dönemde olan hayvanlar için süt SHS'de önemsiz bir azalma gözlendi (p>0.05). Multipar hayvanlar için SHS'nda rakamsal bir azalma mevcut iken; primipar hayvanlar için ikinci aşılamayla birlikte süt SHS'daki düşüşün önemli olduğu saptandı (p<0.01). Ancak, 2. ve 3. aşılamanın sonrasındaki bireysel SHS değerleri benzerdi (p>0.05).

Öneri: Çalışma sonuçları önerilenden farklı bir aşılama protokolü ile mastitis aşısı uygulamasının primipar ineklerde ve tank sütü SHS'nında düşüşe neden olduğu göstermiştir.

Anahtar kelimeler: Aşı, mastitis, somatik hücre sayısı, sütçü inek, tank sütü

Abstract

Aim: This study was conducted to investigate the effect of a mastitis vaccine with non-labelled vaccination regimen on the milk somatic cell count (SCC) in primiparous and multiparous cows at different stages of lactation.

Material and Methods: In the study, 97 healthy Holstein cows were included and the cows were vaccinated on day 0 (d 0), 21 days later (d 21) and 90 days thereafter (d 111). Individual and bulk tank milk samples were collected for SCC analysis on the 30th day prior to the 1st vaccination and on the 30th day after the 2nd and 3rd vaccinations.

Results: Bulk tank milk somatic cell counts (BTMSCC) at pre-vaccination, and after the 2nd and 3rd vaccinations, were 467, 246, and 371 x1000 cell/ml, respectively. It was observed that average milk SCC was numerically decreased after the 2nd and 3rd vaccinations, with respect to pre-vaccination level (p>0.05). Unremarkable declines in individual SCC (iSCC) were observed in the cows at early, mid or late lactation periods (p>0.05). While the decline in iSCC was numerically in the multiparous cows, it was found to be significant after the 2nd vaccination in the primiparous cows (p<0.01). Yet, the iSCC values obtained after the 2nd and 3rd vaccinations were similar (p>0.05).

Conclusion: Results suggested that commercial mastitis vaccine with non-labelled vaccination regimen led to a decline in SCC in the milk, particularly in the primiparous cows and in bulk tank milk.

Keywords: Bulk tank milk, mastitis, vaccine, somatic cell count, dairy cows

Introduction

Mastitis is the inflammation of mammary gland caused by bacteria and causes huge economic losses in dairy farms. These economic costs are mainly due to the reduction of milk production at all stages of lactation, and as the chronically infected cows are culled from the herd (Blowey and Edmondson 2010; Baştan 2013). The primary pathogens that lead to mastitis are *S. aureus, S. uberis, S. dysgalactiae, S. agalactiae, E. coli* and other gram-negative bacteria (Bradley 2002).

Even though standard mastitis control programs reduce the frequency of intramammary infections caused by contagious pathogens (Hillerton et al 1995), due to environmental factors, it is not entirely possible to say that the same holds for clinical mastitis (Lam et al 1997; Barkema et al 1999). Therefore, in order to control both contagious and environmental pathogens, the National Mastitis Council (NMC) prepared a 10-point mastitis control program in 2001 (Sharma et al 2011). Even though 10-point mastitis control programs are applied currently in countries where modern livestock farming techniques are employed, mastitis continues to be a significant problem in dairy farms (Bradley 2002). Therefore, new ways of preventing mastitis have been sought, one of which is vaccination. It is suggested that prevention effectiveness might increase with the use of vaccines especially in the prevention of mastitis due to microorganisms such as *S. aureus* and *E. coli* (Barkema et al 1999; Erskine 2012).

It was reported that vaccination against mastitis could cause positive effects like a reduction in duration and symptoms of coliforms mastitis (Hogan et al 1994; Deluyker et al 2005; Wilson et al 2007), intramammary infection rate (Nordhaugh et al 1994) and somatic cell counts (Leitner et al 2003). However, one of the enormous restrictions in mastitis vaccination is unwieldy vaccination regimen under field condition (Wilson and González 2003).

Our hypothesis was that randomized or non-labelled vaccination could also affect positively udder health in a dairy farm. The objective of the study was to examine the effect of a commercial mastitis vaccine using different vaccination protocol under Turkey field condition on somatic cell counts (SCC) in bulk tank and individual milk in primiparous and multiparous cows at different stages of lactation.

Material and Methods

Animals, housing and management

The study was conducted, at a one location, on a commercial farm located the vicinity of Balikesir, Turkey, where the average daily milk yield was 30 lt/cow; corn silage, clover, hay and concentrated feed, prepared according to the TMR system, were used as rations; the cows were milked three times a day
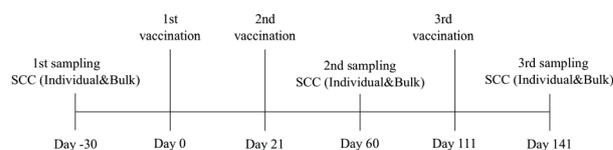

Figure 1. Study design.

using an automatic milking system; and dry therapy and pre- and post-milking teat disinfections were administered. In the farm, all animals were regularly vaccinated (except mastitis) and were routinely subjected to deworming.

Study design

The animals used in the study were 97 Holstein cows between two and six years of age. Cows with each of teat abnormalities/lesions, clinical mastitis, blind quarters or worsening general condition excluded from the study. The study was conducted on-site, randomized without any constraints or criteria. The mixed milk samples from these cows were taken into consideration. Study design was shown in Figure 1.

Within the scope of the study plan, all of the cows were injected with 2 ml of polyvalent inactive mastitis vaccine (Startvac, Hipra Turkey), which contains inactive *E. coli* (J5), *S. aureus* (CP8) SP 140 strain producing slime associated antigen complex (SAAC), liquid paraffin adjuvant and benzyl alcohol, given deep intramuscularly from the neck region after morning milking, collection of milk samples and completion of milking process.

Milk sampling and processing

The milk samples were collected under aseptic conditions, as specified by Harmon et al (1990). Before sampling, the teats were wiped with cotton wool soaked in 70% alcohol, foremilk were taken, and subsequently the mixed milk samples were taken from the udder with a single manipulation, and delivered to the laboratory at +4°C within 24 hours. Milk samples from bulk tank were taken carefully with a sterile dipping vessel from a well-mixed tank and delivered to the laboratory at +4°C.

Laboratory analysis

The somatic cells were counted with an automated fluorescent microscopic somatic cell counter (Bentley IBC-M Bactoscan; Bentley Instruments Inc., Chaska, MN, USA).

Statistical analyses

Prior to significance tests, data were analyzed using the Kolmogorov Smirnov Test, in terms of the normality of distribution. Non-normally distributed data were normalized with logarithmic (Log_{10}) transformation. In order to analyze va-

203

riations in the somatic cell count during the pre-vaccination period, and after the 2nd and 3rd vaccinations, as well as the impact of the period (<100 DIM (early), 101–200 DIM (mid), and 201–305 DIM (late)) and number of lactation on SCC, the method of generalized linear modeling for repeated measures was used. For interactions that were found to be significant, simple effect tests were run. All statistical analyses were examined with minimum 5% margin of error. SPSS® for Windows 14.01 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analyses.

Results

On the classification of the 97 cows examined within the study in terms of the lactation periods, 23 were found to be at early, 39 were found to be at mid, and 35 were found to be at late lactation period; while on the classification of the cows in terms of the parity, 46 were found to be primiparous and 51 were found to be multiparous. It was found that the average days in milk (DIM) for all the cows, were 195.35±12.69, and the mean of lactation number was 2.12±0.08 (X±SE). The descriptive statistics of the findings obtained in the study are given in Table 1 and change in BTMSCC is illustrated in Figure 2 below.

A decline in the BTMSCC was observed following the 2nd and 3rd vaccinations, however it was not statistically significant (p>0.05). While the changes in iSCC of the cows at early, mid and late lactation periods were not statistically significant (p>0.05), the changes in the iSCC in the milk of the primiparous and multiparous cows were statistically different (p<0.01; Table 2).

Even though a decline in iSCC in the milk of multiparous cows was observed, these changes were not significant (p>0.05). For primiparous cows, in turn, a statistically significant decline in iSCC in the milk occurred after the 2nd vaccination (p<0.01); yet, the difference between the SCC in the milk after the 2nd and 3rd vaccinations was not significant (p>0.05; Table 3; Figure 3).

Discussion

Although mastitis vaccines had not been very common in European Union countries until quite recently, nowadays they are sold in many European countries. However, the use of Startvac® (Hipra), which is a polyvalent mastitis vaccine produced against both Enterobacteriaceae and Staphylococcus species, is authorized in the European Union. During authorization activities, this vaccine was reported to reduce the number of new intramammary infections caused by coliform and staphylococcus species, as well as the clinical severity of the disease, but these studies were specifically conducted in the southern regions of Europe (Bradley et al 2015).

While Middleton et al (2009) suggested that the vaccine prepared against S. aureus was not effective in reducing the SCC in milk and the rate of new staphylococcal mastitis, some researchers (Pankey et al 1985; Nordhaug et al 1994; Leitner et al 2003) indicated that the vaccines prepared against S. aureus, reduced somatic cell counts as well as the severity of clinical infections, enabling an effective protection against clinical infections.

Polyvalent vaccines constitute another practice of preventing new infections. Studies have also been conducted in Turkey on vaccines that contain inactive forms of many mastitis agents. While Keskin et al (2007) reported that inactive and polyvalent vaccines were not adequately effective in preventing clinical mastitis, reducing SCC and California Mastitis Test (CMT) scores, Küçük and Alaçam (2003) suggested that polyvalent vaccines were successful in reducing SCC and the rate of S. aureus infections.

Folnožić et al (2014) reported that Startvac® mastitis vaccine was efficacious in controlling clinical mastitis, and found that the BTMSCC declined from 567.200 cells/ml to 177.500 cells/ml within a year following the vaccination. Similarly, the finding of our study indicating a reduction of BTMSCC following the 2nd and 3rd vaccinations, to <400.000 cells/ml from the pre-study level of 467.000 cells/ml, supports

Table 1: Descriptive statistics				
	n	Mean of iSCC (X±SE; x1000 cells/ml)	BTMSCC (x1000 cells/ml)	Percentage of cows with SCC ≥200,000
Pre-vaccination	97	963.87±203.61 (5.20±0.89)*	467	42.27 % (41/97)
After 2nd vaccination	97	614.86±133.75 (5.09±0.85)*	246	39.18 % (38/97)
After 3rd vaccination	97	605.43±177.91 (5.15±0.77)*	371	39.18 % (38/97)

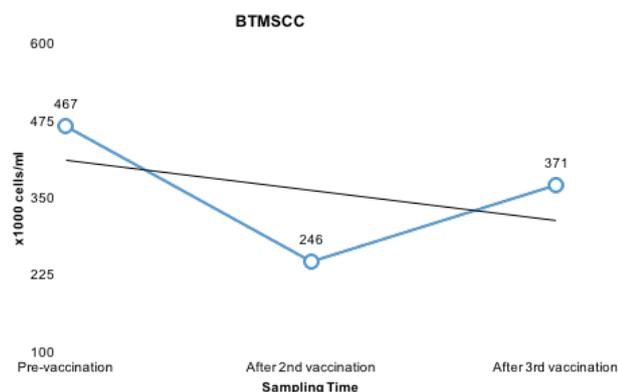* Data obtained after logarithmic transformation.

204

Figure 2. Alteration in BTMSCC

the idea that the vaccine causes a reduction in the SCC. The reduction in SCC following vaccination might stem from a spontaneous recovery in the quarters and a reduction in the severity of inflammation.

March et al (2010) determined that SCC was lower in cows vaccinated with Startvac® when compared with the cows in the control group. The researchers (March et al 2010) applied labelled vaccination procedure whereby the 1st injection was administered 45 days before the possible date of parturition, the 2nd injection was administered 35 days in advance, and the 3rd injection was administered 62 days after the 2nd (i.e. 52 days after parturition). Bradley et al (2015) examined the impact of different administration protocols on the efficacy of the Startvac mastitis vaccine, and indicated that vaccination reduced the clinical severity of the disease considerably, while differences in the administration procedure did not have an effect on SCC.

In our study, on the other hand, the changes in the SCC during

pre-vaccination, and following the 2nd and 3rd vaccinations, were not statistically significant (p>0.05). There are many factors that affect the level and rate of decline in SCC, such as the number of cows with chronic mastitis in the herd, the approach used for cows with chronic mastitis (culling from the herd), and the treatment of the cows with subclinical mastitis. In our study, in turn, neither the cows with subclinical mastitis were treated, nor were the cows with chronic mastitis culled from the herd. Also, taking the duration of the study into consideration, the reason why the decline in the SCC was not statistically significant might be due to such circumstances. Moreover, it should also be considered that the effect of vaccination on the SCC might vary with respect to farm management as well as the vaccination procedure.

Having administered the Startvac mastitis vaccine according to the defined and recommended procedure, Schukken et al (2014) suggested that the vaccination caused a moderate decline in the rate of intramammary infection. The researchers pointed out that when the vaccine is used with other mastitis control programs it could considerably reduce the duration and incidence of intramammary infections. However, in this study the effect of the vaccine on the SCC has not been checked. If the impact of the vaccine on the SCC were checked, a possible decline in SCC could have been detected due to the reduction in the duration of intramammary infections and mastitis incidence.

Another objective of this study was to examine the effect of the vaccine on the iSCC of primiparous and multiparous cows. The outcome of the study indicated that there was a significant reduction in iSCC in primiparous cows with re-

Table 2. Changes in SCC and sources of variation including the interaction between the change in SCC and period and number of lactation.

	SS	d.f.	MS	F-ratio	P value
SCC	0.962	2	0.481	1.097	0.336
SCC*Period	0.522	4	0.131	0.298	0.879
SCC*Parity	5.657	2	2.829	6.453	0.002

SS: Sum of Squares; d.f.: Degrees of Freedom; MS: Mean Squares

Table 3. iSCCs after vaccinations in primiparous and multiparous cows.

Group	n	DIM	Pre-vaccination	After 2nd vaccination	After 3rd vaccination	P
Primiparous	46	230.07±21.30	1040.65±298.16a (5.31±0.13)*	411.63±148.41b (4.91±0.12)*	235.33±50.83b (4.92±0,11)*	**
Multiparous	51	165.94±13.44	894.64±281.10 (5.12±0.12)*	798.16±214.54 (5.25±0.12)*	939.25±329.84 (5.37±0.10)*	NS

a,b: Different letters in the same row indicate statistically significant differences.
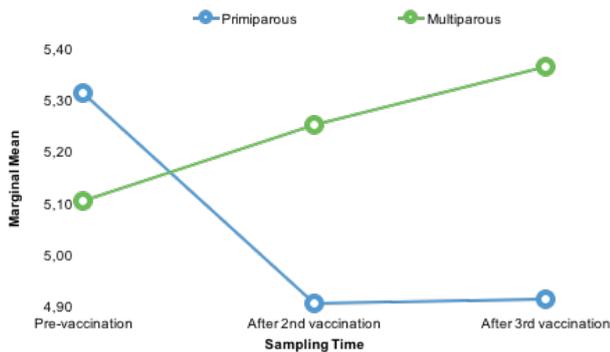*: Data obtained after logarithmic transformation.
**: p<0.01, NS: p>0.05

205

Figure 3. iSCC change in primiparous and multiparous cows

spect to multiparous cows (p<0.01). Parity is an important risk factor for mastitis, and the risk of developing mastitis in older cows is greater than in younger animals. While the exposure of the cow to infection for a longer time, due to the older age, increases the risk of mastitis based on contagious pathogens, the weakening in the teat sphincter muscles in the older cows increases the risk of mastitis, due to environmental pathogens (Baştan 2013). Pantoja et al (2009) recorded that clinical mastitis in multiparous cows almost quadrupled with respect to the previous lactation period. Schukken et al (2014) reported that the efficacy of the vaccination varied according to the age of the animals, as the rate of new intramammary infections caused by *S. aureus* increases with parity, while new intramammary infection risks, due to Coagulase-negative Staphylococci (CoNS) in primiparous animals, are lower. The outcome of our study, indicating that vaccination has been more effective in reducing the SCC of primiparous cows, is in line with the results achieved by the researchers (Schukken et al 2014). The stronger udder immune system in primiparous cows over multiparous cows might be another factor that affects the response to the vaccination.

In this study, whether the vaccination is effective in reducing milk SCC in cows at early, mid or late lactation stage, has been identified (p>0.05). This situation might be related to the dynamic of the infection, and the variation in the pathogen type, according to the lactation period. In addition, it is known that a physiological increase in milk SCC occurs when the udder is worn out, as the stage of lactation advances. An increase in SCC, which may occur even in healthy quarters as lactation stage advances, might be another factor that affects this outcome.

The influence of the vaccine on SCC, with respect to lactation stages, was analyzed in this study but it was established that the effect of the vaccine on the SCC did not vary with respect to the stage of lactation (p>0.05). This outcome is indeed of practical importance, since in the study that examined the efficacy of different vaccination protocols (Bradley et al 2015), the researchers stated that there were certain difficulties in the administration of the standard vaccination

procedure, regardless of the quality of the record keeping at the establishments. The result obtained in our study implied that this polyvalent mastitis vaccine can be used beyond the established protocol.

Accordingly, it was determined that the commercial mastitis vaccine with non-labelled vaccination regimen was more effective in reducing SCC in primiparous cows than in multiparous cows, and that it effectively reduced the BTMSCC (cells/ml).

Conclusion

It can be concluded that the administration of Startvac mastitis vaccine with non-labelled vaccination regimen relatively reduces both individual and bulk tank milk somatic cell counts (p>0.05), whereas the somatic cell counts decline at a more significant rate in primiparous cows (p<0.01). It was thought that non-labelled or randomized vaccination regimens could led satisfactory results. Moreover, it is suggested that conducting new studies on larger groups with different farm conditions would be worthwhile.

Acknowledgements

References

Barkema H, Schukken Y, Lam T, Beiboer ML, Benedictus G, Brand A, 1999. Management practices associated with the incidence rate of clinical mastitis. J Dairy Sci, 82, 1643-54.

Baştan A, 2013. İneklerde Meme Sağlığı ve Sorunları. Kardelen Publ, Ankara, Turkey.

Blowey RW, Edmondson P, 2010. Mastitis Control in Dairy Herds. Cabi.

Bradley AJ, 2002. Bovine mastitis: an evolving disease. Vet J, 164, 116-128, 2002.

Bradley AJ, Breen JE, Payne B, White V, Green MJ, 2015. An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom. J Dairy Sci, 98, 1706-1720.

Deluyker HA, Van Uffel K, Elfring GD, Van Oye SN, Dutton C, Nanhjian I, 2005. Efficacy of a J-5 Escherichia coli bacterin in clinical coliform mastitis dairy cattle, In: Mastitis in Dairy Production, Ed; Hogeveen H, Wageningen Academic Publisher, pp; 285-289.

Erskine R, 2012. Vaccination strategies for mastitis. Vet Clin North Am Food Anim Pract, 28, 257-270.

Folnožić I, Samardžija M, Đuričić D, 2014. Učinak Startvac® cjepiva na kontrolu kliničkog ili subkliničkog mastitisa i

broja somatskih stanica u istočnoj Hrvatskoj. In: Proceedings of XIV Middle European Buiatrics Congress, pp; 116.

Harmon R, Eberhart R, Jasper D, Langlois BE, Wilson RA, 1990. Microbiological Procedures for the Diagnosis of Bovine Udder Infection. National Mastitis Council, Arlington, VA.

Hillerton JE, Bramley AJ, Staker RT, McKinnon CH, 1995. Patterns of intramammary infection and clinical mastitis over a 5-year period in a closely monitored herd applying mastitis control measures. J Dairy Res, 62, 39-50.

Hogan SJ, Weiss WP, Smith KL, Todhunter DA, Schoenberger PS, Sordillo LM, 1994. Effects of an Escherichia coli J5 vaccine on mild clinical coliform mastitis. J Dairy Sci, 78, 285-290.

Keskin A, Seyrek-İntas K, Tek HB, Tuna B, Yılmazbas G, Ozakın C, Ertas S, 2007. Efficiency of polyvalant mastitis vaccine in lactating dairy cows. J Biol Environ Sci, 1, 87-92.

Küçük Ş, Alaçam E, 2003. The effect of udder and milking hygiene on systemic immunization approaches in dairy herds. Ankara Univ Vet Fak Derg, 50, 25-31.

Lam T, Van Vliet J, Schukken Y, Grommers FJ, Velden-Russcher A, Barkema HW, Brand A, 1997. The effect of discontinuation of postmilking teat disinfection in low somatic cell count herds. II. Dynamics of intramammary infections. Vet Quart, 19, 47-53.

Leitner G, Lubashevsky E, Glickman A, Winkler M, Ezra E, Chaffer M, Saran A, Trainin Z, 2003. Development of a Staphylococcus aureus vaccine against mastitis in dairy cows, II. Field trials. J Vet Imm and Immunopath, 93, 151-158, 2003.

Leitner G, Lubashevsky E, Glickman A, Winkler M, Saran A, Trainin Z, 2003. Development of a Staphylococcus aureus vaccine against mastitis in dairy cows: I. Challenge trials. Vet Immunol Immunopathol, 93, 31-38.

March R, Prenafeta A, Noguera M, Guix R, Prenafeta A, 2010. Efficacy evaluation of a new vaccine against bovine mastitis field trial results. In: Proc. NMC Annual Meeting, pp; 286-287.

Middleton JR, Luby CD, Adams DS, 2009. Efficacy of vaccination against staphylococcal mastitis: a review and new data. Vet Microbiol, 134, 192-198.

Nordhaug M, Nesse L, Norcross N, Gudding R, 1994. A field trial with an experimental vaccine against Staphylococcus aureus mastitis in cattle. 1. Clinical parameters. J Dairy Sci, 77, 1267-1275.

Nordhaugh ML, Hesse LL, Norcross LL, Gudding R, 1994. A field trial with an experimental vaccine against Staphylococcus aureus mastitis in cattle. 2. Antibody response. J Dairy Sci, 77, 1276-1284.

Pankey J, Boddie N, Watts J, Nickerson SC, 1985. Evaluation of protein A and a commercial bacterin as vaccines against Staphylococcus aureus mastitis by experimental challenge. J Dairy Sci, 68, 726-731.

Pantoja JCF, Hulland C, Ruegg PL, 2009. Somatic cell count status across the dry period as a risk factor for the development of clinical mastitis in the subsequent lactation. J Dairy Sci, 92, 139-148.

Schukken Y, 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. J Dairy Sci, 97, 5250-5264.

Sharma N, Singh N, Bhadwal M, 2011. Relationship of somatic cell count and mastitis: An overview. Asian-Aust J Anim Sci, 24, 429-38.

Wilson DJ, González RN, 2003. Vaccination strategies for reducing clinical severity of coliform mastitis. Vet Clin North Am Food Anim Pract, 19, 187-197.

Wilson DJ, Mallard BA, Burton JL, Schukken YH, Gröhn YT, 2007. Milk and serum J5-specific antibody responses, milk production change, and clinical effects following intramammary Escherichia coli challenge for J5 vaccinate and control cows. Clin Vaccine Immunol, 14, 693-699.