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Vaccination against mastitis: an overview

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Introduction

Mastitis affects a high proportion of cows throughout the world and is without doubt still one of the most costly diseases for the dairy industry (Bradley, 2002). The financial losses associated with mastitis are mainly incurred by milk production losses, treatment costs, and culling (Huijps et al., 2008). Additionally, farmers supplying milk with high bulk milk somatic cell count may be losing out on bonus payments as well as incurring penalties. Mastitis also accounts for the largest proportion of antibiotic drug use in the dairy industry, strongly harming the image of milk as a high quality product. Indeed, herds with higher bulk milk somatic cell count have a higher risk of antibiotic residue violation because of their increased antibiotic usage (Ruegg and Tabone, 2000). Clinical mastitis has, in addition, its implications for animal welfare (Bradley, 2002). Treating infected cows also increases labor usage (e.g. time and efforts) and causes stress of which the consequences should not be underestimated as they are both perceived as the two most annoying aspects of mastitis by farmers (Jansen et al., 2009).

Among the bacteria that cause bovine mastitis, Escherichia coli (*E. coli*) and Staphylococcus aureus (*S. aureus*) play an important role. Escherichia coli is often involved in hyperacute clinical mastitis cases characterized by abnormal appearance of milk, hard mammary quarters, depressed appetite, reduced milk production, and in worst case scenario dehydration, recumbency, and death. Curative therapy with antibiotics remains only moderately effective and depends on the severity and stage at which the disease is treated. The most successful strategies for preventing and controlling coliform mastitis rely on improving the hygienic management. The severity of clinical symptoms can be reduced by prophylactic immunization with the *E. coli* J5 vaccine (Wilson and Gonzalez, 2003). Despite a shift in the distribution of mastitis pathogens over the year from the more contagious ones towards the more environmental ones, *S. aureus* remains a highly prevalent cause of mastitis worldwide and across many management systems.

Due to the fast transmission from infected to uninfected animals, *S. aureus* intramammary infections are apparently not easy to control and many components of mastitis control programs are necessary to fully control *S. aureus* on dairy farms (Barkema et al., 2006). Such control programs include management procedures such as optimal milking routine, post milking teat disinfection, a well-functioning milking machine, and segregation of known infected animals, culling of long-term affected animals, treatment of infected quarters and the use of dry cow therapy. More recently, the use of vaccines has become an additional tool in the control of *S. aureus* intramammary infections as well (Schukken et al., 2014).

This paper gives an overview of vaccination against mastitis with a focus on the efficacy of vaccination against *S. aureus*.

What is vaccination?

In essence, vaccination is a form of active immunization entailing the introduction of a foreign molecule, e.g. bacteria or parts of the bacteria into the cow causing the cow itself to generate immunity via the production of antibodies specifically oriented against the target. Using this binding mechanism, an antibody can "tag" the bacteria for attack by other parts of the cow's immune system such as macrophages and neutrophils ("opsonization"), or can neutralize its target directly e.g. by blocking a part of the microbe that is essential for either its invasion or survival.

Each vaccine contains a killed or weakened form of the specific organism (e.g. *S. aureus, E. coli*, …) that causes a disease such as mastitis. Even though the organism in the vaccine has been altered so that it won't cause sickness, the part of the organism that stimulates the immune system to respond ("antigen") is still present. Vaccines against *E. coli* primarily contain the inactive J5 *E. coli* strain, resulting in the formation of antibodies against the uniform component lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria causing the severe symptoms associated with hyperacute *E. coli* mastitis cases. Vaccines against *S. aureus* consist of either bacterins (= killed or avirulent/weakened *S. aureus* strains) or exopolysaccharides (= sugar residues secreted by bacteria in the surrounding environment). One of those exopolysaccharides is poly-N-acetylglucosamine (PNAG), a surface polymer produced by a variety of bacterial species, including *S. aureus* and *Staphylococcus epidermidis*, and facilitating bacterial cell-to-cell contact in biofilms.

The mechanism behind vaccination fully relies on the acquired immune response. Vaccination essentially evokes a primary response in which the $CD4^+$ T-lymphocytes and B-lymphocytes play a crucial role. After vaccination, the B-lymphocytes start reacting as if the real infectious organism is invading the body. They start multiplying to form a clone of identical cells that are able to respond to the specific antigen the vaccine contains. The cloned cells subsequently evolve into either plasma cells or memory B-cells. The plasma cells produce antibodies which are trained specifically to attach to and inactivate the organism one is vaccinated against. Over time, the antibody concentration will gradually disappear, but the memory B-cells will remain dormant in the body for a while. The memory B-cells keep a memory of the organism that one was vaccinated against. If one is ever exposed again to identically the same organism, the dormant memory cells will recognize it straight away, and rapidly start multiplying and developing into plasma cells. As the plasma cells are already trained to produce antibodies against the organism, they are able to produce large numbers of antibodies in a short time period. As the antibodies are produced so quickly, they are able to fight the disease even before sickness can occur.

Still, one should keep in mind that the first line cellular immune defense of the mammary gland is determined by the non-specific immunity including the neutrophils and macrophages rather than by the acquired immunity. Lymphocytes, in particular $CD4^+$- and B-lymphocytes seem to be primarily involved in the subacute and chronic phases of mastitis and not in the very early stages of the infection (Sordillo and Streicher, 2002; Grönlund *et al.*, 2006). A long lasting immunological memory against mastitis causing pathogens as described above could yet be induced, neither by the cow nor by vaccination.

What is vaccine efficacy and effectiveness?

Efficacy of a vaccine refers to the reduction in disease measured in a carefully monitored, randomized controlled clinical trial conducted in a homogeneous population according to a defined protocol. In essence, the vaccine efficacy is determined by 4 parameters. The first parameter is the impact of vaccinations on the rate of new infections. This represents the classic vaccine effect, whereby the vaccine reduces the susceptibility of non-infected individuals such that no or fewer infections take place. The second parameter is the impact of vaccination on the infectiousness of an infected individual. The vaccine reduces the amount of shedding of infected but vaccinated individuals compared to non-vaccinated infectious individuals. As *S. aureus* is a mammary pathogen that may be transmitted from cow-to-cow, a reduction in the infectiousness of a vaccinated individual would be valuable. This reduction in infectiousness was also observed in the reported challenge trials (Pérez *et al.*, 2009). The third parameter is the impact of vaccination on the cure of infection. Vaccinations may result in a shorter duration of infection. The duration is essentially the inverse of cure, so a higher cure will result in a shorter duration. The fourth and final parameter of vaccine impact is the reduction in progression of infection from subclinical to clinical mastitis. As clinical mastitis results in milk discard, treatment and animal sickness, a reduction in progression of infection would be of value to the dairy industry. Even though challenge and controlled clinical trials might have shown a certain degree of protection against e.g. *S. aureus* mastitis, the ultimate value, the so-called effectiveness, of the vaccine will always need to be shown under commercial farm conditions. Effectiveness refers to the reduction in disease measured under conditions of use of the vaccine in ordinary clinical practice. The effectiveness is in general somewhat lower than the efficacy.

Vaccines against mastitis

Commercial mastitis vaccines are currently available for immunization against mastitis caused by *S. aureus* and *E. coli*. In the US, there are two *S. aureus* bacterins available. The vaccines are marketed as Somato-Staph® and Lysigin® and are labeled as somatic antigen containing phage types I, II, III, IV and miscellaneous groups of *S. aureus*. There are also 3 coliform mastitis vaccines available. Two of them are identical and marketed as J-5 Bacterin® and Mastiguard® . A separate bacterin-toxoid (J Vac®) is also available. The 4th Gram-negative mastitis vaccine contains re-17 mutant Salmonella typhimurium bacterin toxoid. On the European market, there is only one labeled vaccine against mastitis available (Startvac®). The vaccine contains inactivated *E. coli* (J5), inactivated *S. aureus* (CP8) SP 140 strain expressing Slime Associated Antigenic Complex (SAAC) and adjuvant. The vaccine has a label claim for reducing the incidence of subclinical mastitis and the incidence and severity of the clinical signs of clinical mastitis caused by coliform, *S. aureus*, and coagulase-negative staphylococci (CNS).

At this time, there are no commercial vaccines available that have a proven efficacy against streptococcal mastitis. Still, the increased frequency of mastitis caused by environmental streptococci such as *Streptococcus uberis* has resulted in a number of yet unsuccessful attempts to produce vaccines against these pathogens. The wide strain-variety together with the strain-specific protection in particular slows down the development of vaccines specifically oriented against mastitis causing streptococci (Denis *et al.*, 2009).

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Vaccine effectiveness

A number of studies have been published on the efficacy of vaccination against *S. aureus* mastitis. In one of the first field trials including 30 heifers, a 3-fold decreased risk of *S. aureus* infections was observed in heifers vaccinated with an experimental vaccine formulation based on inactivated *S. aureus* cells and exopolysaccharides at 5w and 1w before calving. Still, no effects on the somatic cell count were found (Giraudo *et al.*, 1997). Nickerson *et al*. (1999) vaccinated heifers with the commercially available Lysigin® at 6 months of age followed by a booster dose 2 weeks later and subsequent vaccinations every 6 months until calving. Vaccinated heifers had a 45% reduction in both new *S. aureus* intramammary infections during pregnancy and new *S. aureus* intramammary infections at calving relative to controls. Middleton et al. (2006) compared the efficacy of the same commercially available vaccine with two experimental formulations and non-vaccinated controls in primiparous heifers as well. Heifers were vaccinated twice, 28 days apart in late gestation with either a 3-isolate experimental bacterin (Group I; n = 11), a 5-isolate experimental bacterin (Group II; n = 11), or the commercially available Lysigin® (Group III; n = 14). Group IV consisted of 11 non-vaccinated control animals. All groups (vaccinated animals and non-vaccinated ones) were challenged with a heterologous strain of *S. aureus* by intramammary infusion on days 6-8 of lactation in a single infection-free mammary quarter. All animals became infected with *S. aureus* after challenge. In contrast to the results obtained by Nickerson and co-workers (1999), no differences in *S. aureus* clearance rates were observed between groups. Animals vaccinated with Lysigin® had a lower mean duration of clinical mastitis and showed less severe symptoms than non-vaccinated control animals. There was no evidence that any of the vaccinated groups had a lower somatic cell count than non-vaccinated control animals, and no evidence that vaccinates had a greater milk yield than controls post-challenge. Still, significantly higher concentrations of milk antibodies against *S. aureus* were observed in the Lysigin® vaccinated animals than in the non-vaccinated control animals (Luby *et al.*, 2007). Similar results were obtained in studies that used avirulent (Pelligrino *et al.*, 2008) or inactivated *S. aureus* (Tenhagen *et al.*, 2001) vaccine formulations or vaccines including insoluble bacterial fragments of two field *S. aureus* field strains and secreted antigens of a third field strain (Leitner *et al.*, 2013). In the latter study, however, the milk somatic cell count was almost 50% lower in the vaccinated animals compared to the non-vaccinated animals. Also, the average daily milk yield was 0.5 kg/day higher in the vaccinated animals than in the non-vaccinated control cows.

In a very recently published study (Schukken *et al.,* 2014), the efficacy of the novel commercially available vaccine Startvac® was evaluated on two commercial dairy farms. In total, 1,156 lactations from 809 cows were enrolled. During the first phase of the trial, all cows that were due to calve were vaccinated until approximately 50% of the cows in the milking herd were vaccinated. At that point, when 50% vaccination coverage was reached, cows that were due to calve were randomly assigned to be vaccinated or left as negative controls. Vaccination of cows was done according to label, with a total of three doses of the vaccine, with the first injection at 45 days before the expected parturition date, the second injection 35 days thereafter (corresponding to 10 days before the expected parturition date), and the third injection 62 days after the second injection (equivalent to 52 days post-parturition). The vaccine efficacy for the rate of new infections was relatively low and depending on the parity. For heifers, the vaccine efficacy for transmission was 25%, indicating that the new infection rate was 25% lower in vaccinated heifers than in non-vaccinated heifers. However, the vaccine efficacy for transmission was still positive but nonsignificant for animals in second lactation (+16%) and even negative (-30%) for animals in third or higher lactation. The vaccine efficacy for cure was moderate with a value of 41%, meaning that the cure rate of *S. aureus* infections in vaccinated animals was 41% higher than in non-vaccinated animals. The latter resulted in a shorter duration of *S. aureus* infections. Still, a significant difference in vaccine efficacy for cure was present between the 2 herds. Combining the transmission parameter and cure rate parameter into the overall basic reproduction ratio, R0, resulted in a value of 0.89 for vaccinated animals and a value of 1.72 for control cows. For CNS, the R0 value for vaccinated animals was 0.91 and 1.40 for control animals. For both CNS and *S. aureus*, vaccination resulted in moving the basic reproduction ratio from above to below the threshold of one. The overall vaccine efficacy was estimated at 45%. In an experimental clinical trial including 8 clinically healthy heifers and cows, four animals were vaccinated with Startvac® at 45 days and 10 days before calving. At 15 days in milk, two contra-lateral quarters of each of the eight cows were inoculated with the formaldehyde killed *S. aureus* C 195 strain (HIPRA, S.A., Amer, Spain) 2 hours after morning milking. The two other quarters were inoculated with phosphate buffered saline (PBS) and served as control quarters. Preliminary results suggest a less severe inflammatory response in vaccinated animals than in non-vaccinated ones. The average daily milk yield per cow was 33.2 liter at the onset of the trial. In the non-vaccinated group average daily milk yield decreased from 34.2 liter/day at 15 DIM to 30.5 liter/day at 16 days in milk. In the vaccinated group, no significant differences in average daily milk yield were observed over time. In both vaccinated and non-vaccinated animals, the quarter milk somatic cell count of the challenged quarters increased over time. The difference in quarter milk somatic cell count between control (42,900 cells/ml) and inoculated quarters (2,079,000 cells/ml) was substantially higher in the non-vaccinated animals compared with the difference in vaccinated animals (88,200 vs 411,500 cells/ml). Interestingly, in the vaccinated group the increase of the quarter milk somatic cell count in the infected quarters was not significantly different from the quarter milk somatic cell count in the control quarters.

The blood concentration of anti-SAAC substantially increased during dry period in the vaccinated animals only. Vaccinated animals had a significantly higher anti-SAAC blood concentration at the time of calving than the non-vaccinated animals. The milk concentration of anti-SAAC from 15 up to 17 DIM was significantly higher in vaccinated animals than in non-vaccinated animals, independently from the infection status of the quarters. The latter findings support the results of Prenafeta *et al.* (2010).

The higher anti-SAAC blood concentrations suggest a more pronounced humoral specific immune response which might explain the shorter duration of the *S. aureus* infections as was found in the study of Schukken *et al.* (2014). Also, the higher anti-SAAC concentrations in milk might potentially trigger the opsonization of the inoculated *S. aureus* bacteria and partly explain why vaccinated animals suffered from a less severe inflammatory reaction than the non-vaccinated animals. In this regard, Camussone *et al.* (2014) immunized 17 pregnant heifers with one of two vaccine formulations composed by either *S. aureus* whole or lysed cells formulated with ISCOM Matrix. Both immunogens induced a strong humoral immune response in blood and milk characterized by a substantial increase in antibody concentration. Neutrophil phagocytosis was much more pronounced in the vaccinated animals than in the non-vaccinated ones, suggesting an increased opsonization of the *S. aureus* bacteria in case of increased antibody concentrations.

Conclusions

The efficacy of vaccination against *S. aureus* is dependent upon the vaccine formulation that is used, the cows' parity, the prevalence of *S. aureus* mastitis at the herd level and the farm management practices that are applied. As concluded by Schukken *et al.* (2014), it seems that on farms with good management practices including excellent milking procedures, antibiotic therapies, and segregation and culling of known persistently infected animals, vaccination will most probably result in a relatively low reduction in new infection rate and a moderate shorter duration of intramammary infections caused by *S. aureus* which might eventually result in an elimination of *S. aureus.* On farms with a poor management, *S. aureus* will most likely show a reduced prevalence but remain endemic despite vaccination. The protection against *S. aureus* by vaccination is most likely the result of an increased opsonization via a vaccine-induced increase in antibody concentrations in blood and milk, facilitating the clearance of *S. aureus* from the mammary gland.

References

Barkema H.W., Schukken Y.H., Zadoks R.N. 2006. Invited Review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. Journal of Dairy Science 89: 1877-1895.

Bradley, A.J. 2002. Bovine mastitis: an evolving disease. The Veterinary Journal 163: 1-13.

Camussone C., Veaute C.M., Pujato N., Morein B., Marcipar Iván. 2014. Immune response of heifers against *Staphylococcus aureus* CP5 whole cell and lysate vaccine formulated with ISCOM Matrix adjuvant. Research in Veterinary Science 96: 86-94.

Denis M., Wedlock D.N., Lacy-Hulbert S.J., Hillerton J.E., Buddle B.M. 2009. Vaccines against bovine mastitis in the New Zealand context: What is the best way forward? New Zealand Veterinary Journal 57:132-140.

Giraudo J.A., Calzolari H;, Rampone H., Rampone A., Giraudo A.T., Bogni C., A. Larriestra, R. Nagel. 1997. Field trials of a vaccine against bovine mastitis. 1. Evaluation in heifers. Journal of Dairy Science 80:845-853.

Grönlund U., Johannisson A., Persson-Waller K. 2006. Changes in blood and milk lymphocyte sub-populations during acute and chronic phases of *Staphylococcus aureus* induced bovine mastitis. Research in Veterinary Science 80:147-154.

Huijps, K., Lam T.J.G.M., Hogeveen H. 2008. Costs of mastitis: facts and perception. Journal of Dairy Research 75: 113-120.

Jansen, J., van den Borne B.H.P, Renes R.J., van Schaik G., Lam T.J.G.M., Leeuwis C. 2009. Explaining mastitis incidence in Dutch dairy farming: The influence of farmers' attitudes and behaviour. Preventive Veterinary Medicine 92: 210-223.

Leitner G., Pinchasov Y., Morag E., Spanier Y., Jacoby S., Eliau D., Pitcovski J. 2013. Immunotherapy of mastitis. Veterinary Immunology and Immunopathology 153:209-216.

Luby C.D, Middleton J.R., Ma J., Rinehart C.L., Bucklin S., Kohler C., Tyler J.W. 2007. Characterization of the antibody isotype response in serum and milk of heifers vaccinated with *Staphylococcus aureus* bacterin (LysiginTM). Journal of Dairy Research 74:239-246.

Middleton J.R., Ma J.N., Rinehart C.L., Taylor V.N., Luby C.D., Steevens B.J. 2006. Efficacy of different Lysigin (TM) formulations in the prevention of *Staphylococcus aureus* intramammary infection in dairy heifers. Journal of Dairy Research 73:10-19.

Nickerson S.C., Owens W.E., Tomita G.M., Widel P. 1999. Vaccinating dairy heifers with a *Staphylococcus aureus* bacterin reduces mastitis at calving. Large Animal Practice 20:16-28.

Pellegrino M., Giraudo J., Raspanti C., Nagel R., Odierno L ;, Primo V., Bogni C. 2008. Experimental trial in heifers vaccinated with *Staphylococcus aureus* avirulent mutant against bovine mastitis. Veterinary Microbiology 127:186-190.

Pérez, M.M., Prenafeta A., Valle J., Penadés J., Rota C., Solano C., Marco J., Grilló M.J., Lasa I., Irache J.M., Maira-Litran T., Jiménez-Barbero J., Costa L., Pier G.B., de Andrés D., Amorena B. 2009. Protection from *Staphylococcus aureus* mastitis associated with poly-N-acetyl beta-1,6 glucosamine specific antibody production using biofilm-embedded bacteria. Vaccine 27: 2379-2386.

Piepers S., De Meulemeester L., de Kruif A., Opsomer G., Barkema H.W., De Vliegher S. 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. Journal of Dairy Research 74:478-483.

Piepers S., De Vliegher S., Demeyere K., Lambrecht B., de Kruif A., Meyer E., Opsomer G. 2009. Technical note: flow cytometric identification of bovine milk neutrophils and simultaneous quantification of their viability. Journal of Dairy Science 92:626-631.

Prenafeta A., March R., Foix A., Casals I., Costa L. 2010. Study of the humoral immunological response after vaccination with a *Staphylococcus aureus* biofilm-embedded bacterin in dairy cows: possible role of the exopolysaccharide specific antibody production in the protection from *Staphylococcus aureus* induced mastitis. Veterinary Immunology and Immunopathology 134: 208-217.

Ruegg PL., Tabone T.J. 2000. The relationship between antibiotic residue violations and somatic cell counts in Wisconsin dairy herds. Journal of Dairy Science 83: 2805-2809.

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 In Press.

Sordillo L.M., Streicher K.L. 2002. Mammary gland immunity and mastitis susceptibility. Journal of Mammary Gland Biology and Neoplasia 7:135-146.

Tenhagen B.A., Ediger D., Baumgartner B., Kalbe P, Klunder G., Heuwieser W. 2001. Efficacy of a herd-specific vaccine against *Staphylococcus aureus* to prevent postpartum mastitis in dairy heifers. Journal of Veterinary Medicine A 48:601-607.

Van Oostveldt K., Vangroenweghe F., Dosogne H., Burvenich C. 2001. Apoptosis and necrosis of blood and milk polymorphonuclear leukocytes in early and midlactating healthy cows. Veterinary Research 32:617-622.

Wilson D.J., Gonzalez, R.N. 2003. Vaccination strategies for reducing clinical severity of coliform mastitis. Veterinary clinics of North America – Food Animal Practice 19: 187-197.