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Estimation of efficacy of Startvac® vaccination in dairy herds

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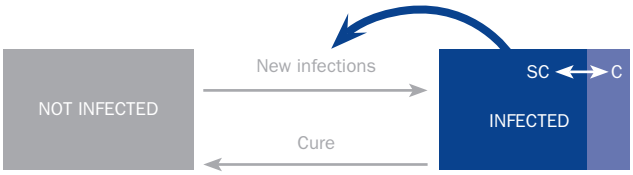
Introduction

Among the bacteria that cause bovine mastitis, *Staphylococcus aureus* (*S. aureus*) plays an important role. Many infections of the mammary gland are due to this pathogen and the role of *S. aureus* in mastitis is worldwide and across many management systems. The control of *S. aureus* intramammary infections is apparently not easy 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, 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. This is especially valuable as antibiotic treatment of intramammary infections has come under scrutiny. Cell surface polysaccharides have been proposed as vaccine candidates. One of these carbohydrate antigens, poly-N-acetylglucosamine (PNAG), is a surface polymer produced by a variety of bacterial species, including *S. aureus* and S. epidermidis. PNAG is an adhesin that facilitates bacterial cell-to-cell contact in biofilms. It was recently shown that bacterins from strong biofilm-producing *S. aureus* bacteria triggered the highest production of antibodies to PNAG and conferred the highest protection against infection and mastitis following intra-mammary challenge with biofilm-producing *S. aureus* bacteria. Thus, bacterins from strong biofilm bacteria were used to develop a vaccine against *S. aureus* ruminant mastitis.

Even thought challenge trials have shown a certain degree of protection of bacterins agains the *S. aureus* challenge, the ultimate value of the vaccine will need to be shown under commercial farm conditions. Estimation of vaccine efficacy under field conditions is therefore essential. However, estimation of vaccine efficacy is complex and it is important to fully understand the potential components of vaccine efficacy that may be affected by the vaccine under consideration. In figure 1, four components of the infectious process that may be affected by a vaccine are shown in a simplified schematic. The first component is the impact of vaccinations on the rate of new infections. This represents the classic vaccine effect, whereby the vaccine reduces the susceptibility of not infected individuals such that no or fewer infections take place. The second component 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 component 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 component 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.

To evaluate vaccine efficacy of a *S. aureus* vaccine under field conditions, all four components of vaccine efficacy should be evaluated and preferably quantified separately. The design and analysis of vaccine evaluation studies has been the topic of many recent studies, and progress in this field of science allows the execution of field trials that are able to provide insight in most if not all component of vaccine efficacy.
In this paper, the design of a field trial for the estimation of vaccine efficacy of a new *S. aureus* vaccine will be discussed and the first preliminary results will be presented.

Figure 1. Schematic representation of the infectious processes where vaccination may play a role. Four processes are represented: susceptibility to new infections, infectiousness, cure of infection and progression to clinical disease.



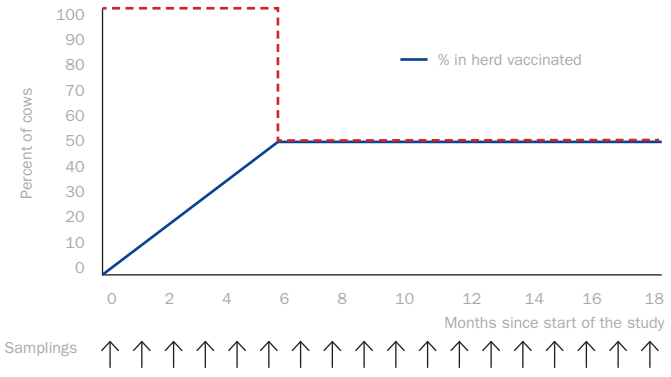
Study design

The study to estimate vaccine efficacy was a randomized negative control field trial, whereby animals in two herds were randomly assigned to either vaccination or no-treatment controls. The two dairy herds were selected based on herd size (approximately 500 lactating cows in total), known prevalence of *S. aureus*, ability to keep records, participation in dairy herd improvement monthly test day measurements and the willingness and interest of the owners to participate in the study. One of the herds was overseen by staff of Università degli Studi di Milano, the other herd was overseen by the herd's private practitioner (FT).

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 full immunization program was repeated with each gestation. Both pregnant heifers and cows in lactation 1 and higher were included in the trial.

Vaccination took place according to the design shown in Figure 2. For the first 6 months, all heifers and cows in late gestation were vaccinated. After 6 months, or until approximately 50% of animals in the herd had been enrolled in the vaccination program, vaccination was done on only 50% of animals.

Figure 2. Design of a within herd randomized controlled trial to estimate the efficacy of a *S. aureus* vaccine.



By vaccinating all animals for the first 6 months, the objective of 50% vaccination was reached as fast as possible. After the initial 100% vaccination period, true randomization happened thereafter. This design allows us to evaluate vaccine efficacy starting 6 months into the study. The herds will be followed for an additional 12 months after the first period of 100% vaccination of cows in late gestation. The vaccine contains inactivated Escherichia coli (J5); inactivated *Staphylococcus aureus* (CP8) SP 140 strain expressing Slime Associated Antigenic Complex (SAAC) and adjuvant. The vaccine is administered intramuscularly. The vaccine has a label claim for reducing the incidence of sub-clinical mastitis and the incidence and the severity of the clinical signs of clinical mastitis caused by coliform, *S. aureus* and coagulase negative staphylococci. In this report we will focus on the efficacy of the vaccine against *S. aureus* only.

Sampling of all quarters of all lactating cows takes place on a monthly interval. Also, cows that have calved, dried-off, have a case of clinical mastitis or cows that are being removed from the herd are samples by herd personnel. On all samples a somatic cell count will be measured. All samples are cultured at the mastitis laboratory of Università degli Studi di Milano. All *S. aureus* and CNS isolates are frozen for further analyses. For all bacterial species, and approximate colony count will be performed. At the completion of the study, it is expected that approximately 40,000 samples will have been collected.

The ultimate outcome of the study will be an estimate of vaccine efficacy. Vaccine efficacy for susceptibility is calculated as: VE_s = 1 - Relative risk of infection in vaccinated versus controls. Similarly, the vaccine efficacy for cure is: VE_c = 1 - Relative risk of the duration of infected in vaccinated versus control. The vaccine efficacy for infectiousness and progression to clinical can be calculated.

By using a within herd randomized controlled design, vaccinated and controls cows will be comparable with regard to all housing, environment and management variables with the exception of their vaccination status. This allows for a valid comparison of vaccinated and controls. The disadvantage of such a design is the bias towards no-effect that is inherent in such a design. Because non vaccinated control cows are partly protected by their vaccinated herd mates, they will show a lower incidence of infection. At the same time, the vaccinates are exposed to more infectious material due to the fact that they are surrounded by non-vaccinated herd mates. Hence, control are less exposed and likely less infected, while vaccinates are more exposed and likely more infected compared to a situation that the whole herd was either not vaccinated or fully vaccinated. As a result the difference between vaccinated and controls is likely smaller compared to a comparison of fully

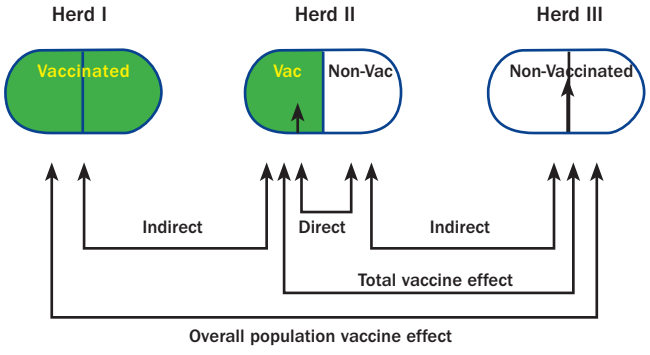
vaccinated and fully non-vaccinated herds. The difference in infection risk in a within herd randomized vaccination trial is called the direct vaccine effect. The difference in infection risk in non-vaccinated animals between a fully non-vaccinated herd and a randomized vaccinated and control herd is called the indirect vaccine effect. The sum of these two effects is called the total vaccine effect. A pictorial summary of these vaccine effect estimates is shown in figure 3. The comparison of a fully vaccinated and a fully non-vaccinated herd will allow the calculation of the overall population vaccine effect. The latter estimate is the most relevant vaccine effect when vaccinations are applied to populations of animals rather than to individual animals. Depending on the vaccine and the vaccine usage on a farm, the direct vaccine effect of the overall population vaccine effect will be the most valid estimate for a specific vaccine.

The precise field study as developed for the Startvac® vaccine will eventually allow the calculation of all four vaccine efficacy estimates (susceptibility, cure, infectiousness and progression). To allow for a correction of the direct vaccine effect for the bias towards no effect, a mathematical modeling approach will be used to obtain an unbiased estimate of vaccine efficacy. To be able to obtain an unbiased estimate, the risk of new infections in the vaccinated and non-vaccinated control population will be modeled as:

$$\text{New infections}_v = \beta_v \,.\, \#\text{negativev} \,.\, \#\text{positive}_{v+c}$$

$$\text{New infections}_c = \beta_c \,.\, \#\text{negative}_c \,.\, \#\text{positive}_{c+v}$$

Figure 3. Study designs for vaccine efficacy estimation and the relevant vaccine effects for each study design.



The number of new infections is modeled as a function of a transmission parameter, multiplied by the number of culture negative quarters and the number of positive *S. aureus* shedding quarters. In these equations, v is for vaccinates and c is for non-vaccinated controls. The unbiased vaccine efficacy (VE) for susceptibility can then be calculated as:

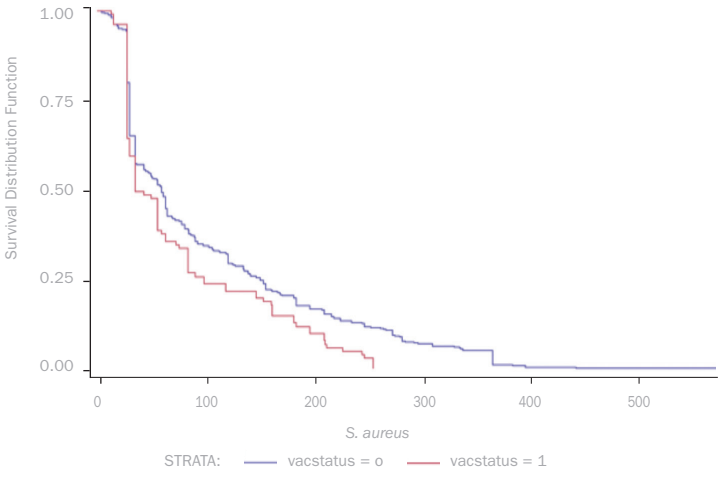
$$VE = 1 - \frac{\beta_v}{\beta_c}$$

Preliminary results

The randomized controlled field trial is approximately halfway it full length. Cows have been vaccinated for about one year and in both herds the vaccination schedule has now changed to a 50%/50% allocation of vaccinated and controls. In both herds, data is of high quality with very few missing values. Prevalence of *S. aureus* in the herd is approximately 10%, while the prevalence of coagulase negative staphylococci is approximately 5%. These relative high prevalences indicate that sufficient challenge is present in both herds.

The initial results during the first months of the valid comparison of vaccinates and controls after the start of the randomized 50%/50% vaccination schedule shows a lower incidence of new *S. aureus* infections in vaccinated animals versus control animals. These initial data show a vaccine efficacy for susceptibility of approximately .50 or 50%. No difference between vaccinated and controls is observed in average colony forming units in S. aureus infected cows. However, the average duration of infection of a S. aureus infection is shorter in the vaccinated animals compared to the non-vaccinated control animals. The difference in duration of infectious period is shown in Figure 4. A first estimate of vaccine efficacy of cure was calculated as .73 or slightly over 70%. These initial estimates of vaccine efficacy for *S. aureus* are based on relative small numbers and need to further confirmed during the remaining months of the study.

Figure 4. Time to cure or end of observation period for S. aureus infections in either vaccinated cows (red line) or non-vaccinated control cows (blue line).



Discussion and conclusions

Estimation of vaccine efficacy of contagious mastitis organisms under field conditions is an interesting challenge. The design of a randomized controlled trial is even more complicated if vaccination is limited to late gestation so that the number of vaccinated individuals increases only slowly over time. Vaccine efficacy has at least four components and intensive longitudinal studies are necessary to be able to estimate the four different components of vaccine efficacy. Ultimately all these four components will contribute to the success of a vaccine, whether measured in infection dynamics in a population or in the economic benefit of vaccination.

An intensive and large randomized field trial to evaluate the efficacy of Startvac® vaccination is described in detail. The study is currently underway and only initial estimates of vaccine efficacy can be provided. The first results indicate an acceptable vaccine efficacy for susceptibility and for cure of infection. However, several months of additional data are essential to further confirm and stabilize the initial estimates of vaccine efficacy. When the final efficacy estimates are available, further economic modeling will be possible to define the cost-benefit ratio of the Startvac® vaccination program.

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