Biofilm prevention from the start

STARTVAC®
Inactivated mastitis vaccine against E. coli, S. aureus, coliforms and coagulase-negative staphylococci.

HIPRA Symposium
Results of Mastitis Vaccination
WBC’12, Lisbon
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Since its beginnings HIPRA has always been true to the philosophy and vision that defines the company: TO BECOME THE REFERENCE IN PREVENTION FOR ANIMAL HEALTH. To do so, we have invested heavily in researching and developing products that improve the health, performance and well-being of production animals. HIPRA has a wide range of vaccines for preventing and controlling diseases affecting livestock and continues working to find innovative solutions to promote continuous improvement in animal health worldwide.

Mastitis is the most important disease in the dairy cattle sector and causes significant economic losses in all farms as well as being a problem for animal welfare and which can also result in an overuse of antimicrobials. We are aware of the importance of prevention measures in controlling this disease and the need for finding new tools to improve the results obtained thus far.

Therefore, in line with our commitment to animal health and as the result of years of development, we are proud to present today the efficacy results for STarTVaC®.

STarTVaC® is the first and only vaccine registered by the European Medicines Agency (EMA) that prevents new infections by *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative staphylococci and coliforms while reducing the severity of mastitis, decreasing consumption of antibiotics and lowering individual somatic cell counts. STarTVaC® prevents biofilm formation because it contains the necessary technology to induce antibodies that slow the development of the layer of biofilm-producing strains of *Staphylococcus aureus*.

Thus, we thought the best way to show you our vaccine is to present its qualities and experiences via the studies that will be presented by Sofie Piepers from the University of Ghent and Ynte Schukken from Cornell University, with the aim of making available to veterinarians a reliable, useful and effective tool that can become an integral part in the control of this disease.
Bacterial biofilm

Biofilms are a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adhesion to an inert or living surface (Costerton et al., 1999). This can constitute a protected niche that allows bacteria to grow and survive in a hostile environment, particularly in environments characterized by a continuous flow. When biofilms are formed in live sheath environments, they are generally more sensitive to mechanical breakage. In addition to protection against physical and chemical environmental agents, the biofilm provides extracellular catalysis and the concentration of nutrients on cell surface. In more natural environments, microorganisms try to adhere to available surfaces. Hence, the free-swimming (planktonic) phase can be viewed as a bacterial dispersal from one surface to another another. Thus, the initial phase of biofilm formation involves two stages: the first one consists in attachment of cells to a surface, facilitated by cell wall associated adhesins, which are produced in large numbers (Mack, 1999). Attachment to native polymeric surfaces is increased in the presence of matrix proteins including fibronectin, and fibrinogen. Following initial attachment of cells to a surface, the primary cell aggregates produce extracellular adhesives to facilitate clumping.
Immunological response to an experimental intramammary inoculation with a killed Staphylococcus aureus strain in vaccinated and non-vaccinated lactating dairy cows

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Introduction

 Mastitis accounts for the largest proportion of antibiotic drug use in the dairy industry (Heremans et al., 2000). Ongoing political debates and public concerns about the emergence of antimicrobial resistance and drug resistance in milk stress the need for alternatives to antibiotic therapy. In particular, the prophylactic use of antimicrobials is coming under scrutiny. One such use of antibiotics is dry cow therapy. As a consequence, there is an increasing interest in the possibilities to boost the host immune responses.

The objective of this study was to unravel the innate immunological response after administration of a novel vaccine (Startvac®, HIPRA, S.a., Amer, Spain), containing the inactivated Escherichia coli J5 strain and the Staphylococcus aureus SP 140 strain expressing slime-associated antigenic complex (SaaC) (Prenafeta et al., 2010). The aim of this study was to evaluate the effect of administration of the Startvac® vaccine (HIPRA, S.a., Amer, Spain) on milk PMN concentration and viability. Secondly, the production of the antigen-specific antibodies anti-SaaC (against S. aureus) and anti-J5 (against E. coli) in blood was determined over dry period.

Materials and Methods

Eight clinically healthy cows and heifers were selected at the research dairy farm of the Faculty of Veterinary Medicine, Ghent University, Belgium (Agri Vet). Three animals were vaccinated intramammarily at 45 days and 10 days before the expected calving date with the Startvac® vaccine (HIPRA, S.a., Amer, Spain) containing the inactivated Escherichia coli J5 strain and the Staphylococcus aureus SP 140 strain expressing slime-associated antigenic complex (SaaC) (Prenafeta et al., 2010). At 15 days in milk (DIM), two contralateral quarters of each of the six cows were inoculated with the formaldehyde killed Staphylococcus aureus C 195 strain (HIPRA, S.a., Amer, Spain) 2 hours before morning milking. The two other quarters were inoculated with phosphate buffered saline (PBS) and served as control quarters. Duplicate quarter milk samples (5 ml) were aseptically collected for bacteriological culturing and leukocyte differential count and viability analysis at 2 hours before, and at 4, 12, 24 and 48 hours after challenge. During the entire trial bacteriological culture and somatic cell count of the milk of all four quarters was frequently evaluated, this to exclude interference with naturally occurring intramammary infections. In conclusion, vaccinated cows seem to develop a less severe inflammatory reaction after inoculation compared to non-vaccinated animals. Vaccination also increased the level of the antigen-specific antibodies anti-SaaC and anti-J5 in blood which might eventually result in a shorter duration of the infection. However, further research is definitely needed before final conclusions on the impact of prepartum vaccination on the cows’ innate immune response and their udder health status shortly after calving can be drawn.

Keywords: mastitis, vaccine, immunity

Results of Mastitis Vaccination

The tanks vaccinated and non-vaccinated evaluated were set up as a Latin square, with a two-contralateral quarter design of cow. Two tanks of four milk samples were collected at 45 and 10 days before calving and at 15 days after calving just before the infection is induced. Quarter milk samples are collected at 2 hours before, and at 4, 12, 24 and 48 hours after challenge. During the entire trial bacteriological culture and somatic cell count of the milk of all four quarters was frequently evaluated, this to exclude interference with naturally occurring intramammary infections. In conclusion, vaccinated cows seem to develop a less severe inflammatory reaction after inoculation compared to non-vaccinated animals. Vaccination also increased the level of the antigen-specific antibodies anti-SaaC and anti-J5 in blood which might eventually result in a shorter duration of the infection. However, further research is definitely needed before final conclusions on the impact of prepartum vaccination on the cows’ innate immune response and their udder health status shortly after calving can be drawn.

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Both heifers and multiparous cows suffer from immune suppression around parturition, characterized by a higher proportion of less viable blood and milk polymorphonuclear leukocytes (PMN) (Van Oostveldt et al., 2007) and performed at the lab of the Mastitis and Milk Quality Research Unit (Merelbeke, Belgium). Quarter milk SCC (qSCC) was quantified by electronic counting (Direct Cell Counter, De Laval, Gent, Belgium).

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The milk used to isolate PMN was divided into several 50 ml Falcon tubes and diluted 1:1 with PBS. All tubes were centrifuged (600×g) during 15 minutes, the cream layer and supernatant were removed, and each pellet was suspended into 10 ml PBS. Two pellets were mixed together and again centrifuged (200×g) during 10 minutes, this was repeated two more times. Subsequently, milk PMN were differentiated from other milk cells by a two-step fluorescent immunolabeling using a primary anti bovine monoclonal granulocyte antibody (CH138A) (VMRD Inc., Pullman, WA, USA) and an Alexa 647 labeled goat anti mouse IgM secondary antibody (Molecular Probes, Invitrogen, Netherlands) as previously described (Piepers et al., 2009). To identify apoptotic and necrotic PMN, a double fluorescence isothiocyanate/propidium iodide (FITC-annexin-V (Roche, Indianapolis, IN, USA) and propidium iodide (PI) (Sigma-Aldrich, Bornem, Belgium) staining was used. PMN that were positive for FITC and negative for PI were considered as ‘early’ apoptotic whereas PI that were positive for both FITC and PI were considered necrotic. Polymorphonuclear neutralophilic leukocytes that were negative for both stains were considered viable (Piepers et al., 2009; Van Oostveldt et al., 2012).

Table 1: Sample overview

<table>
<thead>
<tr>
<th>Days before calving</th>
<th>Days into milk</th>
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<tbody>
<tr>
<td>Tasks</td>
<td></td>
</tr>
<tr>
<td>Vaccination</td>
<td>45d 10d</td>
</tr>
<tr>
<td>Challenge</td>
<td></td>
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<tr>
<td>Collection of milk samples:</td>
<td></td>
</tr>
<tr>
<td>- Somatic cell count</td>
<td></td>
</tr>
<tr>
<td>- Bacterial culture</td>
<td></td>
</tr>
<tr>
<td>- PMN</td>
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</tbody>
</table>

1 Three of the six cows were vaccinated.
2 Polymorphonuclear neutrophils.
The concentration of the antigen-specific antibodies anti-SaAC and anti-J5 in blood was determined as previously described (Prenafeta et al., 2010).

Linear mixed regression models adjusting for clustering of repeated measurements within quarters as well as for clustering of quarters within cows were fit to evaluate the association between the cows’ vaccination status before calving and the evolution of qSCC, milk PMN concentration (Log10 PMN), and milk PMN viability (expressed as the proportion of viable PMN), respectively, in both the inoculated and control quarters. A similar model was fit to evaluate the association between vaccination at 45 and 10 days before calving and the evolution of qSCC, milk PMN concentration (Log10 PMN), and milk PMN viability (expressed as the proportion of viable PMN), respectively, in both the inoculated and control quarters (Figure 2).

Results and Discussion

All animals remained clinically healthy during the trial period. Challenge did not affect clinical parameters such as heart beat rate, respiration rate, and mucosal consistency or appetite. The average body temperature 2 hours before inoculation was 38.6°C and 38.8°C for the vaccinated and nonvaccinated animals, respectively, and did not significantly differ between both groups. In both groups, body temperature slightly increased between 15 and 17 DIM.

The average daily milk yield (MY) per cow was 33.1 L at the onset of the trial. In the non-vaccinated group average daily MY decreased from 32.3 L/ day at 15 DIM to 27.5 L/day at 18 DIM (P = 0.06). In the vaccinated group, no significant differences in average daily MY were observed over time. In both groups of animals, the qSCC of the challenged quarters increased over the study period. The difference in qSCC between the control and inoculated quarters was substantially higher in the non-vaccinated animals compared with difference in vaccinated animals (P = 0.001). Interestingly, in the vaccinated group the increase of the qSCC in the infected quarters was not significantly different from the qSCC in the control quarters (P = 0.21) (Figure 3). The preliminary results for qSCC and milk and milk PMN viability in the control quarters are consistent with other studies (Nickerson et al., 1999; Middleton et al., 2000). The difference in PMN viability in control and control quarters during the trial period did not depend on the vaccination status of the animal.

The blood concentration of both anti-SAAC and anti-J5 substantially increased during dry period in the vaccinated animals only (P < 0.05). Vaccinated animals had a significantly higher anti-SAAC and anti-J5 blood concentration at the time of caking than the non-vaccinated animals (P < 0.05) (Figure 4 & 5).

Conclusions

Based on these preliminary results, vaccinated cows seem to undergo a less severe inflammatory reaction after inoculation compared to nonvaccinated animals. This could possibly explain why no change in daily MY was observed in the vaccinated animals, while the non-vaccinated animals suffered from a substantial drop in milk production in the days after challenge. The higher anti-SAAC and anti-J5 blood concentration might result in a more pronounced humoral specific immune response and thus eventually in a shorter duration of the infection. Further research is definitely needed before final conclusions can be drawn.

Acknowledgement

The authors want to thank Lars Hüppe (Department of reproduction, Obstetrics, and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium) for his excellent technical assistance.

References


Estimation of efficacy of Startvac® vaccination in dairy herds

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Cornell University

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 (Barberena et al. 2006). Such control programs include management procedures such as optimal milking routine, post milking heat 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 capsular antigens, polysaccharide glycosaminoglycan (PGN), is a surface polymer produced by a variety of bacterial species, including S. aureus and S. epidermidis. PGN is an adhesion 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 S. aureus intramammary infections. This is especially valuable for the control of S. aureus due to this pathogen and the role of S. aureus in mastitis is worldwide and across many management systems.

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.
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 vaccines and c is for non-vaccinated controls. The unbiased vaccine efficacy (VE) for susceptibility can then be calculated as:

$$\text{VE} = 1 - \frac{c}{v}$$

**Preliminary results**

The randomized controlled field trial is approximately halfway its 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 50%, 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 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 infection 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.

**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.

### References


STARTVAC® Inactivated vaccine. Bovine mastitis, in injectable emulsion. COMPOSITION PER DOSE (2 ML): Inactivated Escherichia coli (J5) 50 RED60*; Inactivated Staphylococcus aureus (CP8) SP 140 strain expressing SAAC** RED80***. Adjuvant. * RED 60: Rabbit effective dose in 60% of the animals (serology). **SAAC: Slime Associated Antigenic Complex. *** RED80: Rabbit effective dose in 80% of the animals (serology).

PROPERTIES: Mastitis is one of the main problems in dairy cows, not only from an economic point of view due to losses in the quantity and quality of the milk, but also from a sanitary point of view, because the milk produced has low bacteriological quality and a high level of antibiotics, as a consequence of antimastitis treatments. The vaccine STARTVAC, which combines specific antigens and a special adjuvant, prevents and minimizes the effects of mastitis caused by Staphylococcus aureus (the main responsible for chronic mastitis) and Escherichia coli (causative agent of acute clinical mastitis).

INDICATIONS: Cows and Heifers: To prevent Mastitis. For herd immunization of healthy cows and heifers, in dairy cattle herds with recurring mastitis problems, to reduce the incidence of sub-clinical mastitis and the incidence and the severity of the clinical signs of mastitis caused by Staphylococcus aureus, coliforms and coagulase-negative staphylococci. The full immunisation scheme induces immunity from approximately day 13 after the first injection until approximately day 78 after the third injection (equivalent to 130 days post-parturition).

SIDE EFFECTS: Slight to moderate transient local reactions may occur after the administration of one dose of vaccine, which disappears within 1-2 weeks at most. ADMINISTRATION ROUTE: Intramuscularly into the neck muscles. The reactions should be preferably administered on alternate sides of the neck. It is advisable to administer the vaccine at a temperature between +15 and +25 °C. SHAKE before use.

DOSAGE: Cows and Heifers: 2 ml/animal. Generally, the following vaccination programme is recommended: First injection at 45 days before the expected parturition date. Second injection: 35 days thereafter (corresponding to 10 days the expected parturition date). Third injection: 62 days after the second injection (corresponding to 130 days the expected parturition date). Post injection: 62 days after the second injection (corresponding to 130 days the expected parturition date). In some cases, the vaccine may be used during pregnancy and lactation.

WITHDRAWAL PERIOD: 0 days. SPECIAL PRECAUTIONS: Store at +2 to +8 °C, avoiding freezing. Protect from light. PACKAGING: Pack of 20 vials of 1 ds. 5 ds vial. 25 ds bottle. Under veterinary prescription. Marketing authorisation holder: Laboratorios Hipra, S.A. la Selva, 135, 17170 AMER (Girona) SPAIN. Marketing authorisation numbers: 1 dose: (EU/2/08/092/003); 5 doses: (EU/2/08/092/002). Use medicines responsibly.