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Report on multifocal assays for the development of the STARTVAC® vaccine according to a new protocol

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1. Introduction

Vaccination with the STARTVAC® vaccine aims to improve the quality of milk from dairy cow farms. This vaccination induces the production of antibodies against certain biofilm components of *Staphylococcus aureus* and *Staphylococcus spp.* and against LPS, an endotoxin of the cell wall of *Escherichia coli*. This dual effect not only reduces the prevalence of mammary infections caused by *Staphylococcus spp.* but also the severity of the clinical signs which accompany infections caused by *E. coli*.

The MA dossier puts forward a vaccination protocol with three injections given around the dry period of cows (RCP STARTVAC®). The disadvantage of this protocol is that the

animals must be vaccinated individually, based on the estimated date of calving. As such, it would be much more practical to vaccinate the entire herd on the same day, with boosters given at regular intervals. For this reason, we tested an alternative vaccination protocol in a field study: all the animals from a herd were vaccinated at the same time on D1, D21 and D111, regardless of their physiological stage. To maintain this level of protection, boosters were given every 3 months following the three initial injections.



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2. Materials and Methods

Dairy farms (n=10, between 40 and 100 dairy cows, and production levels between 8,000 and 14,000 kg, a total of 531 lactating cattle) of a good technical level were included in the study. For these farms the rearing conditions, such as milking facilities and animal housing, are shown in Figure 1.

Dairy cows and heifers were vaccinated for the first time in May-June 2010, with boosters on Day 21 and Day 111. For 3 out of 10 farms it was not possible to vaccinate the future first-calvers at the same time as the cows. Their vaccination protocol began upon their return to the cowshed to prepare for calving.

At the same time as the first vaccination, the milk quality inspection was conducted in order to identify risk factors in each farm before the start of the study. (The results of these inspections are shown in Figure 2).

During the study, new mammary infections were monitored by conducting bacteriological tests on all cows with cell counts greater than 200,000 cells/ml (included in May 2010 and January and May 2011) and of the milk from cows with clinical mastitis. The cell counts of the milk tank were also monitored throughout the study (Figure 3).

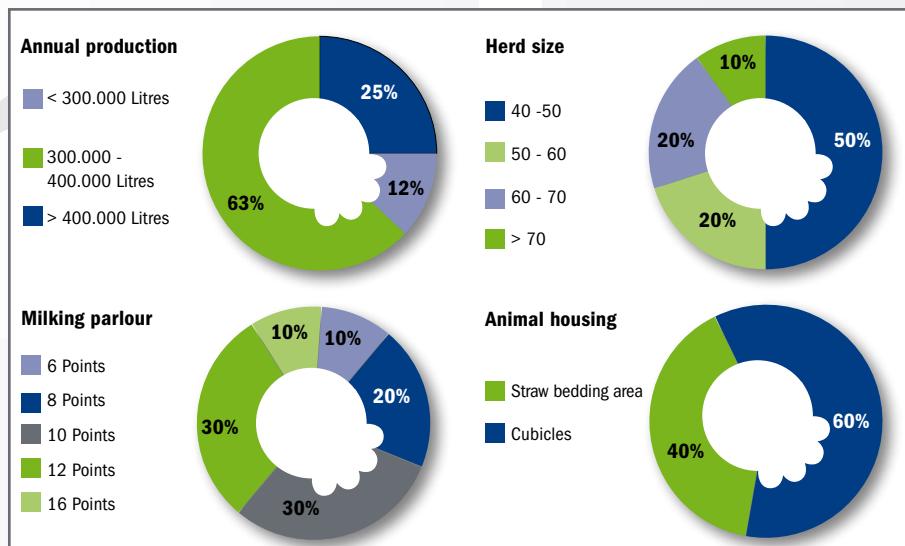


Figure 1. Production, herd size, milking facilities and animal housing of the farms included in the study.

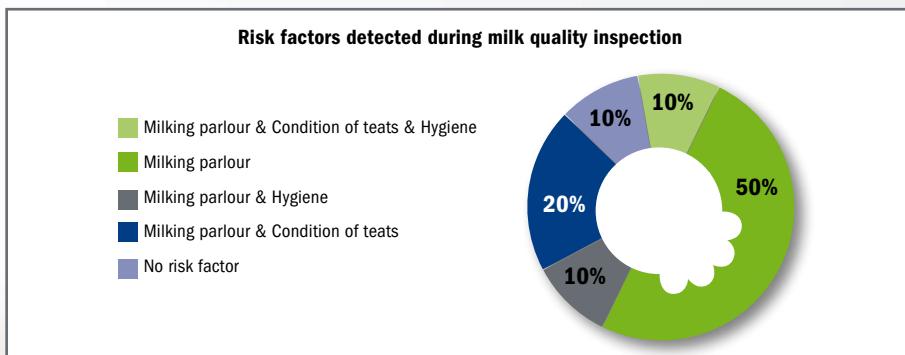


Figure 2. Results of the milk quality inspections made during vaccination.

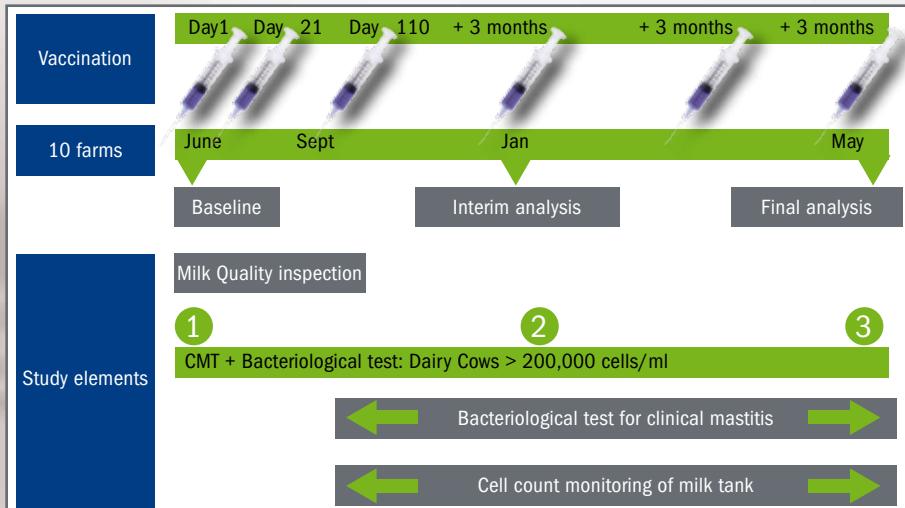


Figure 3 : Study protocol

3. Results and Discussion

In implementing the vaccination protocol, only 2 in 531 cattle showed an increase in temperature (up to 39.6 °C) the day following the injection. The vaccination caused neither pain nor injury at the injection site, nor did it detract from the animals' level of production.

Overall, the percentage of clinical mastitis, as well as the cell counts of the milk tank, decreased from 136% to 55% and from 308,000 to 227,000 cells/ml, respectively, between May 2010 and May 2011 (Figure 4).

Mastitis rates decreased in 9 out of 10 farms: the remaining cases of mastitis were primarily caused by *Streptococcus uberis* and *Coagulase-negative staphylococci* (CNS) (Figure 5).

After vaccination, the average cell counts of the milk tank dipped below 250,000 cells/ml in 7 out of 10 farms, compared with only 4 farms before vaccination. Before vaccination, *Staphylococcus aureus* was found in samples from cows with sub-clinical mastitis in 9 out of 10 farms. After vaccination, *Staphylococcus aureus* was found in only 3 out of 10 farms (Figure 6).

Sub-clinical infections after vaccination were primarily caused by Coagulase-negative staphylococcal infections (Figure 6). It is interesting that the decrease in cell counts in the milk tank is not accompanied by a decrease in the number of infected cows, but by a lower number of infected quarters per cow (Figure 7).

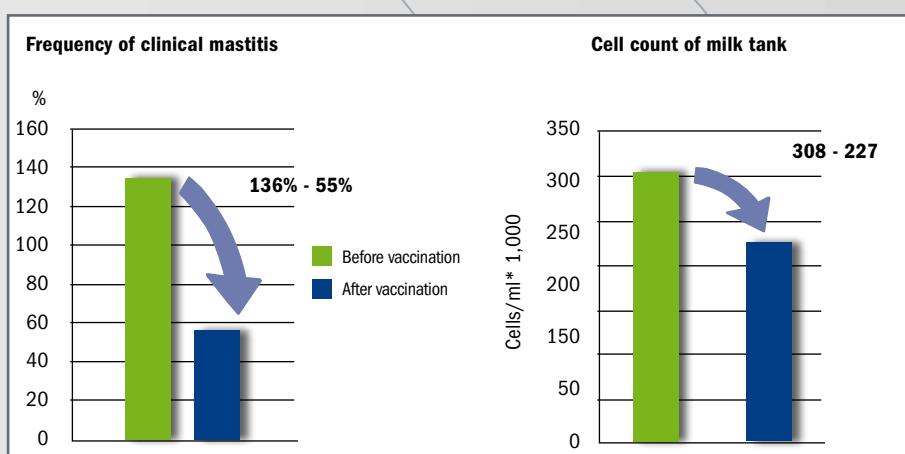


Figure 4. Frequency of mastitis and cell counts of milk tanks on the farms before and after vaccination.

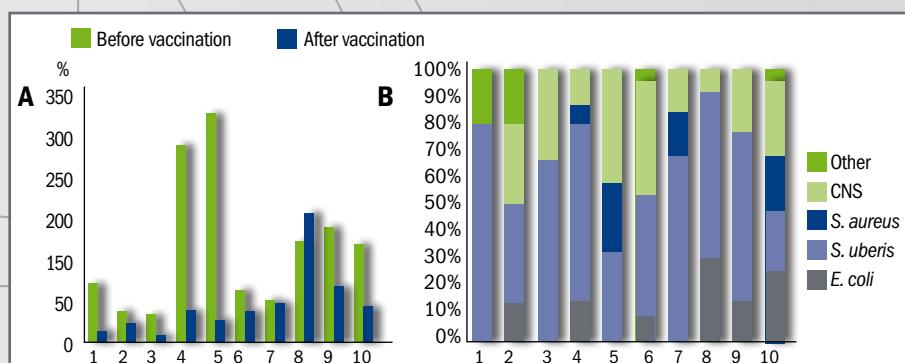


Figure 5. A. Frequency of clinical mastitis before and after vaccination (percentage relative to the number of dairy cows present). B. Aetiology of clinical mastitis observed after vaccination with STARTVAC®.

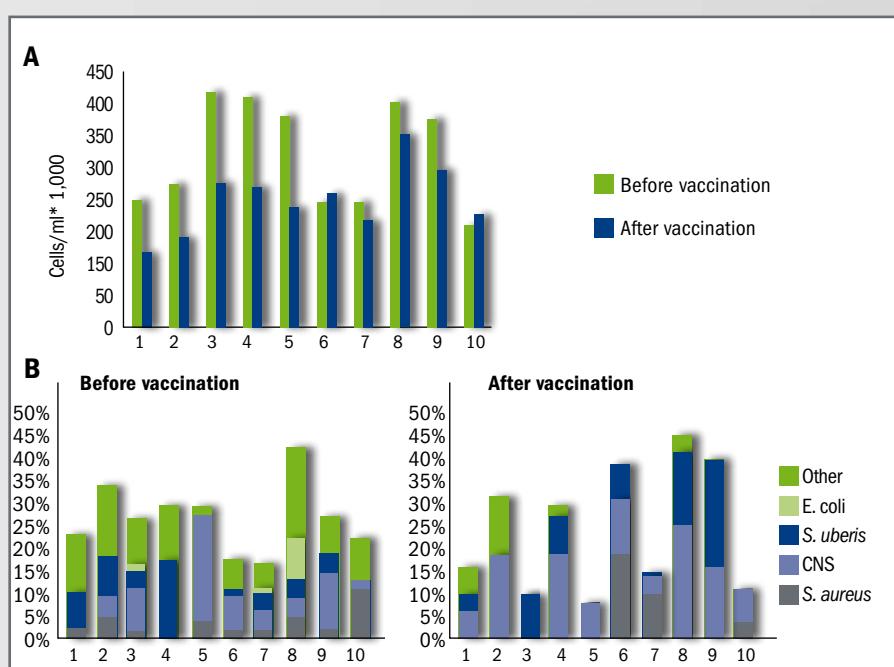


Figure 6. A. Average annual cell counts in the milk tanks of the 10 farms before and after vaccination. B. Aetiology of sub-clinical infections before and after vaccination.

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4. Conclusion

Although vaccination with STARTVAC® does not eliminate the presence of *E. coli* and *S. aureus* in livestock, the rate of clinical mastitis and cell counts of the milk tank decreased in the majority of the farms. After vaccination, clinical mastitis was primarily caused by *Streptococcus uberis* and *Coagulase-negative staphylococci*, while sub-clinical infections were caused by *Coagulase-negative staphylococci*.

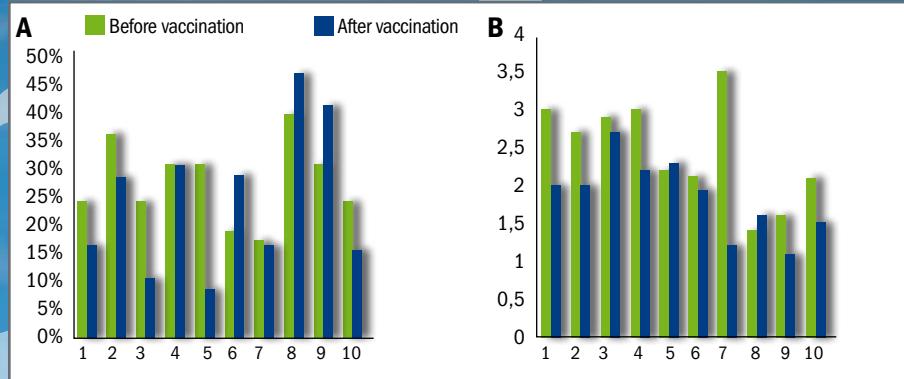


Figure 7. A. Percentage of cows with sub-clinical infections (> 200,000 cells/ml) at baseline and in January 2011. B. Number of infected quarters per cow (positive California Mastitis Test) at baseline and in January 2011.



STARTVAC Inactive vaccine. Before mastitis. In injectable emulsion. COMPOSITION PER DOSE (2 mL): Inactivated Escherichia coli US, 50 REDg⁺; Inactivated *Escherichia coli* (CDC) SP, 140, strain expressing SAAc⁺; 50 REDg⁻; Adjuvant, 4% REDg⁻. Rabbit effective dose in 60% 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 immunisation 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/or severity of the clinical signs of bovine mastitis caused by *Staphylococcus aureus*, coliforms and coagulase-negative staphylococci. **ADMINISTRATION:** full immunisation schedule: 1st injection: 45 days before the expected parturition; 2nd injection: 35 days after the first injection (equivalent to 10 days post-parturition); 3rd injection: 62 days after the second injection (equivalent to 25 days post-parturition). The full immunisation programme should be repeated with each gestation. The whole herd should be immunised. Immunisation has to be considered as one component in a complex mastitis control program that addresses all important udder health factors (e.g. milking technique, dry-off and breeding management, hygiene, nutrition, bedding, cow comfort, air and water quality, health monitoring) and other management practices. Can 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/2/08/092/004); 25 doses: (EU/2/08/092/006). Use medicines responsibly.

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