Case study: insights from *Staphylococcus aureus* antibody monitoring in milk on a 1000 cow herd

SRM Koralagamage[1], PJ Aitken[2], A Pernthaner[1]
[1]Koru Diagnostics, Palmerston North; [2]Totally Vets, Palmerston North

Introduction

Infections of the mammary gland with *Staphylococcus aureus* are common in dairy herds worldwide. In many countries, *S. aureus* is the main cause of subclinical mastitis, leading to elevated somatic cell counts (SCC) and reduced milk quality. The infection is contagious, and the cycle is maintained by undiagnosed chronically infected animals (Rainard *et al.* 2018). Control of *S. aureus* is hindered by the ineffectiveness of therapeutic approaches, especially during lactation (Barkema *et al.* 2006), and by the difficulty of identifying infected cows. Ultimately, the interplay between the cow-specific immune response to the infections and the genetic makeup of the pathogen determines the characteristics of the infection.

A key feature of an infection with *S. aureus* is the highly variable bacterial shedding. Sears *et al.* (1990) found that even infections with the same specific *S. aureus* strain resulted in diverse shedding patterns, with some cows being non-shedders over extended periods of time, while others almost continuously shed the pathogen in high numbers. This finding demonstrates that cow-specific factors have a major effect on shedding patterns. On the other hand, *S. aureus* possesses an array of defence mechanisms to evade immune surveillance and the damaging effects of an immune response, enabling long-term survival in the mammary gland. For example, *S. aureus* can protect itself from host responses by forming biofilms, causing intracellular infections, or expressing Protein A on the surface to evade antibody-based immune defence (Zecconi and Scali 2013; Goldmann and Medina 2018).

The diagnosis of persistent or subclinical infections using methods relying on direct pathogen detection is notoriously difficult. For instance, for bacterial culture to achieve a sensitivity of >95%, two or even three samples are required, significantly increasing the cost of diagnosis (Zecconi, 2010). Novel diagnostic tests with high diagnostic sensitivity that are simple to use are therefore important for establishing programs that effectively control the infection.

The production of *S. aureus*-specific antibodies is an essential part of the host-specific immune response to an infection. Leitner *et al.* (2000) revealed the association between chronic *S. aureus* mammary gland infection and the resulting specific IgG response in serum and milk, indicating that IgG is a suitable biomarker for infection. A range of other immunoassays that predominantly detect specific antigens have been developed for ELISA, as well as lateral flow tests (reviewed by Fabres-Klein *et al.* 2014), demonstrating that the use of IgG to detect *S. aureus* infections is widely accepted.

ELISA tests are cheaper than PCR or culture tests. ELISAs that detect specific antibodies are likely to be less affected by irregular shedding than PCR tests, as antibodies persist even if bacteria are not present in the milk. Thus, ELISA tests may have an advantage over PCR and culture tests for screening herd test samples in both cost and sensitivity.

We developed an IgG ELISA that can also be performed on herd test samples. We employed this test to obtain information on prevalence, persistence, and the effect on milk production of the

infection, as well as the success rate of dry cow therapy (DCT) in a longitudinal study on a 1000-cow herd.

Material and methods

This study was conducted on a 1000-cow herd located in the Manawatu region of New Zealand, where an increase in bulk tank milk SCC (BMSCC) was observed at the end of the 2021/22 and again in the 2022/23 lactation. Such an increase in SCC is indicative of contagious mastitis, and an infection with *S. aureus* was suspected.

Testing for *S. aureus*-specific IgG antibodies was conducted using a commercially available ELISA test (StaphGold). Antibody data were expressed as the test sample to positive ratio (S/P) and, based on given cutoffs, regarded as antibody positive (Pos), suspect positive (Sus), or negative (Neg). Routine herd test (HT) samples from the entire herd were collected at the end of the 2022/23 lactation (HT4 23), less than four weeks before dry-off. All cows were dried off using antibiotic DCT and teat seal.

The herd was re-tested for the presence of S. aureus-specific IgG in milk three times during the 2023/24 lactation: at the first (HT1 24), second (HT2 24), and fourth (HT4 24) herd test time points. S. aureus-specific antibody data were used to calculate the proportion of antibody-positive cows at any given time point. This data was then utilized to determine cure rates (defined as HT4 23 antibody-positive cows returning antibody-negative results at the HT1 24 and HT2 24 testing points), identification of chronic infections (antibody-positive at HT4 23, HT1 24, and HT2 24 or HT4 24), new infections (antibody negative at HT1 24, positive at HT2 24 and/or HT4 24), and reinfections (antibody positive at HT4 23, negative at HT1 24 and HT2 24, and positive at HT4 24). Antibody data were also compared to somatic cell count (SCC) and milk production (kgMS) at herd testing time points. A statistical analysis is currently being conducted.

Results and discussion

During the 2022/23 lactation, the percentage of cows with high SCC (>150,000) rose from 16% to 41%, indicating a potential infection with a contagious mastitis pathogen. Examination of the herd at HT4 23 revealed a high percentage of cows testing positive or suspected positive for *S. aureus* antibodies in both low SCC (79%) and high SCC cows (85%). This discovery, combined with the escalating SCC, justified implementing blanket dry cow therapy. While the prevalence of *S. aureus* infections and low shedding in low SCC cows and cows with a subclinical infection is well established (Wald et al., 2029), the high prevalence of antibody-positive cows at end of the lactation suggests a more complex interaction between host and pathogen than previously understood. The New Zealand pasture-based system with synchronized calving likely facilitates infection spread. Additionally, cows at various lactation stages may display differing susceptibility levels, transitioning from a stronger inflammatory T Helper (Th)1 type response early on to a pronounced tolerogenic Th2 type response later, designed to protect the foetus (Wang *et al.* 2020). The shift from a Th1 to a Th2 type response likely to increases susceptibility to *S. aureus* infections (Rainard *et al.* 2022), especially given the similar lactation statuses of cows, which may explain the high proportions of antibody-positive cows observed.

Re-assessment of the herd for *S. aureus*-specific antibodies at HT1 of the subsequent lactation showed a cure rate (absence of specific anti *S. aureus* antibodies) of 65% for low SCC cows and 55% for high SCC cows, indicating differences in disease progression or infection timing between the two groups, with recent infections more common in low SCC cows.

Monitoring antibody dynamics over time provided insights into infection persistence and dynamics. During the following lactation, 23% of initially HT4 antibody-positive low SCC cows that remained infected over the dry period transitioned to high SCC cows, with an additional 11% at HT2, indicating infection progression correlated with an increase in inflammatory markers as measured by SCC.

Tracking *S. aureus*-specific antibodies alongside SCC data allowed for defining infection status and dynamics, identifying treatment-resistant or chronically infected cows. This enabled informed decision making by the supporting veterinarian and farmer on interrupting the chain of infection. Establishing a separate, smaller herd of *S. aureus* antibody-positive cows, combined with selective culling, reduced SCC increase and antibody-positive cow prevalence observed previously, demonstrating that the control program was partly effective.

Moreover, beyond elucidating the complex interaction between SCC and specific antibodies, antibody-positive cows exhibited reduced performance. Milk production, expressed as kg MS at herd testing points, of antibody positive cows was significantly lower than that of antibody negative cows.

Acknowledgment

We are grateful to the owner of the herd for his consent to mine Infovet and Minda data files and to use herd test milk samples for this work. We also thank Dr R. Thresher from LIC for providing defatted herd test samples and Dr Suzanne Hurst for reviewing the MS and helpful discussions.

References

Barkema H, Schukken Y, Zadoks R. 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, 2006

Fabres-Klein MH, Aguilar AP, Silva MP, Silva DM, Ribon AOB. Moving towards the immunodiagnosis of staphylococcal intramammary infections. *European Journal of Clinical Microbiology & Infectious Diseases* 33: 2095-2104, 2014

Goldmann O, Medina E. Staphylococcus aureus strategies to evade the host acquired immune response. I*nt J Med Microbiol*.308(6): 625-630, 2018

Leitner G, Yadlin B, Glickman A, Chaffer M, Saran A. Systemic and local immune response of cows to intramammary infection with Staphylococcus aureus. *Research in Veterinary Science* 69: 181-184, 2000

Rainard P, Foucras G, Fitzgerald JR, Watts J, Koop G, Middleton J. 2018. Knowledge gaps and research priorities in Staphylococcus aureus mastitis control. *Transboundary and emerging diseases* 65*:* 149-165, 2018

Rainard P, Gilbert FB, Germon P. Immune defenses of the mammary gland epithelium of dairy ruminants. *Frontiers in Immunolology* 13: 1031785, 2022

Safak R, Risvanli A, Asci-Toraman Z. Th1 / Th2 cytokine polarization in milk according to different pathogens causing subclinical mastitis in cows. *Mljekarstvo* 72 (2): 105-113, 2022

Sears PM, Smith BS, English PB, Herer PS, Gonzalez RN. Shedding Pattern of Staphylococcus aureus from Bovine Intramammary Infections. *Journal of Dairy Science* 73: 2785-2789, 1990

Wald R, Hess C, Urbantke V, Wittek T, Baumgartner M. Characterization of Staphylococcus Species Isolated from Bovine Quarter Milk Samples. *Animals (Basel)* 9(5): 200, 2019

Wang W, Sung N, Gilman-Sachs A, Kwak-Kim J. T Helper (Th) Cell Profiles in Pregnancy and Recurrent Pregnancy Losses: Th1/Th2/Th9/Th17/Th22/Tfh Cells. *Frontiers in Immunology* 11: 2025, 2020

Zecconi A. Staphylococcus aureus mastitis: What we need to know to control them. *Israel Journal of Veterinary Medicine* 65: 93-99, 2010
Zecconi A, Scali F. Staphylococcus aureus virulence factors in evasion from innate immune defenses in human and animal diseases. *Immunological Letters* 150(1-2): 12-22, 2013