Symposium review: Features of *Staphylococcus aureus* mastitis pathogenesis that guide vaccine development strategies*

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ABSTRACT

Bovine mastitis affects animal health and welfare and milk production and quality, and it challenges the economic success of dairy farms. *Staphylococcus aureus* is one of the most commonly found pathogens in clinical mastitis but it also causes subclinical, persistent, and difficult-to-treat intramammary infections. Because of the failure of conventional antibiotic treatments and increasing pressure and concern from experts and consumers over the use of antibiotics in the dairy industry, many attempts have been made over the years to develop a vaccine for the prevention and control of *Staph. aureus* intramammary infections. Still, no commercially available vaccine formulation demonstrates sufficient protection and cost-effective potential. Multiple factors account for the lack of protection, including inadequate vaccine targets, high diversity among mastitis-provoking strains, cow-to-cow variation in immune response, and a failure to elicit an immune response that is appropriate for protection against a highly complex pathogen. The purpose of this review is to summarize key concepts related to the pathogenesis of *Staph. aureus*, and its interaction with the host, as well as to describe recent vaccine development strategies for prevention and control of *Staph. aureus* mastitis.

Key words: bovine mastitis, *Staphylococcus aureus*, pathogenesis, vaccine

INTRODUCTION

For decades now, an important amount of significant knowledge and research data have been collected about mastitis in dairy cows. This research has been driven by the increasing need to find effective ways to detect,

control, and prevent the disease. Although mastitis can occur following udder trauma or some physiological disorders, it is most often due to bacterial IMI. Despite great progress in our comprehension of host–pathogen interactions, the use of new farm management practices, and the development of novel treatment and prevention tools, bovine mastitis is still the most common disease with the greatest economic impact for the dairy industry (Bar et al., 2008; Ruegg, 2017; Aghamohammadi et al., 2018).

An IMI can manifest in clinical forms of variable threat levels, according to the severity of the symptoms. An IMI can also manifest with a total absence of visible, macroscopic signs of disease, in the form of a subclinical infection. The clinical or nonclinical character of mastitis is mainly influenced by the genus and species of the responsible pathogen (Rainard and Riollet, 2006; Zadoks et al., 2011). Subclinical IMI are responsible for most of the economic problem associated with mastitis (Shim et al., 2004; Petrovski et al., 2006). The vast majority of IMI cases in herds occurs in the subclinical form, and *Staphylococcus aureus* is an important contributor to such subclinical IMI (Reyher et al., 2011).

Treatment and prevention of *Staph. aureus* IMI using antibiotic therapy is difficult. Reported cure rates for *Staph. aureus* mastitis have been shown to vary considerably and seem to depend on many factors at the herd, cow, and bacterial levels, such as herd transmission rates, cow parity, level of SCC, and the genetic background and phenotypic traits of *Staph. aureus* isolates, including their level of biofilm production (Barkema et al., 2006; Ster et al., 2017). Moreover, persistence of the pathogen in the gland can last the lifetime of the animal (Sutra and Poutrel, 1994). Early treatment of new infections can be effective; however, chronically infected cows generally respond poorly to treatment during lactation. In fact, conventional approved treatments rarely achieve a cure rate greater than 20 to 50% and, most of the time, there is a relapse of infections during subsequent lactations, demonstrating that the pathogen is still present or that a new infection can

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arise easily (Dinsmore, 2002; Roy and Keefe, 2012; Ster et al., 2017). In general, extended antibiotherapies or treating dry cows are strategies with a higher probability of cure (Roy and Keefe, 2012; Rainard et al., 2018). There are also risks associated with antibiotherapy, and the public pressure to reduce antibiotic use in animal production is an important incentive to find efficient nonantibiotic control measures for this disease. Of particular concern are the spread of antibiotic resistance genes by horizontal transfer (Li and Zhao, 2018) and the emergence of livestock-associated methicillin-resistant *Staph. aureus* (MRSA) strains that can be transmitted from cows to humans (García-Álvarez et al., 2011; Holmes and Zadoks, 2011).

Thus, preventing infection through vaccination would be a judicious choice for the control of *Staph. aureus* IMI. Ideally, vaccination would eliminate the reservoir of infection in herds, reduce the incidence of new cases of IMI, and improve milk production and quality. Unfortunately, the versatility of the pathogen, which produces a variety of virulence factors, its numerous ways of camouflaging itself from the immune system, and the diversity of strains that can actually cause the disease have prevented successful vaccine development in recent decades (Ismail, 2017; Rainard et al., 2018). However, advances made in the understanding of *Staph. aureus* pathogenesis and on how the host immune system operates during *Staph. aureus* infections open the door to further progress. Figure 1 summarizes host–*Staph. aureus* interactions and provides an overview of the topics of discussion reviewed here, notably the complexity of *Staph. aureus* pathogenesis and some vaccine development strategies.

EFFECT OF *STAPH. AUREUS* IMI

Several families of bacteria can cause mastitis in dairy cows. Overall, staphylococci (*Staph. aureus* and non-*aureus* staphylococci) are the most common mastitis-causing agents, followed by streptococci and *Escherichia coli* (Contreras and Rodriguez, 2011; Reyher et al., 2011; Condas et al., 2017). Pathogens causing IMI can be broadly classified into 2 epidemiological categories: contagious bacteria and environmental bacteria. Coliform bacteria are often described as noncontagious environmental pathogens associated with strong clinical symptoms (Hogan and Smith, 2003). On the other hand, contagious pathogens are mostly transmitted during the milking process and a reservoir of such bacteria can exist within herds. As such, there is real potential that these pathogens cause outbreaks with high incidence rates (Murai et al., 2014). The contagious pathogens include *Staph. aureus*, non-*aureus* staphylococci, *Streptococcus* spp., *Mycoplasma* spp., and

Corynebacterium bovis. *Staphylococcus aureus* is one of the most important causes of the disease worldwide and the most commonly isolated pathogen in the context of both clinical and subclinical IMI in Canada (Reyher et al., 2011; Naqvi et al., 2018). Milk from infected cows is the main source of transmission of microorganisms from infected cows to their healthy neighbors and this mostly occurs during milking (Olde Riekerink et al., 2008). There is a strong link between transmission of the disease and management quality of the milking facility, which includes teat disinfection procedures and the general cleanliness of the milking equipment used.

Subclinical IMI represent a specific concern because they can stay unnoticed for some time, contribute to the infectious reservoir, and thus increase the risk of transmission. Subclinical IMI can be suspected following an important increase in SCC in milk and confirmed by bacteriology or molecular tests that detect the presence of microorganisms in milk. *Staphylococcus aureus* can alter various milk constituents through production of various hydrolytic enzymes and, consequently, reduce the yield and quality of milk products. The total economic losses associated with *Staph. aureus* clinical and subclinical IMI result from the decrease in milk production and quality, the costs of antibiotic treatments and veterinary fees, and the withdrawal period of cows treated with antibiotics (Halasa et al., 2007; Olde Riekerink et al., 2008; Aghamohammadi et al., 2018).

PATHOGENESIS OF *STAPH. AUREUS* IMI

Several important elements are involved in the virulence of *Staph. aureus* that allow the pathogen to adapt to the mammary gland environment. The pathogenesis of *Staph. aureus* in bovine mastitis has been detailed in several reports over the years (Sutra and Poutrel, 1994; Middleton, 2008; Rainard et al., 2018), so we will summarize here some of the key steps from which vaccine targets can be extrapolated.

Three specific phases are involved in the *Staph. aureus* infection process: (1) adhesion to host cell and extracellular matrix, (2) invasion or penetration into tissues and cells, and (3) evasion of the host immune system (Middleton, 2008). This process is associated with the differential expression of a variety of virulence factors at the different stages of infection. In *Staph. aureus*, the expression of virulence factors is under the control of global regulators such as Agr (accessory gene regulator) and the alternative transcriptional sigma factor SigB (Novick and Geisinger, 2008).

The SigB factor is involved in the response to different stresses and promotes the expression of adhesins and biofilm formation for colonization (Horsburgh et al.,

auto-inducing peptide (AIP), resulting from the coordinated activity of AgrD and AgrB, accumulates in the extracellular milieu and eventually reaches a critical concentration to act on the 2-component system AgrC and AgrA to allow expression of a regulatory RNA (**RNAIII**) that is able to significantly reduce surface protein expression (Novick and Geisinger, 2008). The RNAIII regulator acts directly at the translational level by repressing adhesins as well as *rot* (repressor of tox-

ins) mRNA, and the latter is, in contrast, a repressor of exotoxin synthesis and activator of adhesin synthesis (Boisset et al., 2007). Furthermore, RNAIII permits the translation of *hla* mRNA to allow production of the well-known *Staph. aureus* α -hemolysin, which can lyse several host cell types.

The dynamic regulation of virulence factors orchestrated by the Agr system and other regulators modulates the different phases of *Staph. aureus* IMI. The teat canal represents an important physical barrier that *Staph. aureus* encounters during milking before it is able to spread to the entire mammary gland (Rainard and Riollet, 2006). Following this intrusion, the first step in the colonization of the mammary gland is bacterial adhesion to the host epithelial cells and their extracellular matrix. This attachment allows the bacteria to resist evacuation from the pressure of the milk flux. *Staphylococcus aureus* expresses many virulence factors that are involved in adhesion, including fibronectin (**FnBP**), fibrinogen (**FgBP**) and collagen (CNA) binding proteins, as well as clumping factors (Clf) A and B, and teichoic acids (Brouillette et al., 2003; Mitchell et al., 2008; Middleton et al., 2009). Many such factors are known as MSCRAMM (microbial surface components recognizing adhesive matrix molecules). Biofilm formation is also important in the colonization phase (Otto, 2013). A biofilm is a self-produced extracellular polymeric matrix that can protect the community of bacteria against the action of antibiotics and host immune responses (Hathroubi et al., 2017). After this adhesion phase, *Staph. aureus* synthesizes and secretes factors that allow the invasion, penetration, and destruction of the mammary tissue, including several exotoxins (hemolysins and leukocidins) and various hydrolytic enzymes such as proteases, coagulase, lipases, and hyaluronidases (Middleton, 2008; Suriyaphol et al., 2009). The hemolysins and exoenzymes are implicated in the degradation of the epithelium of the cistern, duct, and alveoli. These microlesions in the mammary gland tissue are responsible for the reduction of milk production, even in the case of subclinical mastitis, when the infection remains unapparent.

Finally, *Staph. aureus* produces a range of factors that allow it to escape but also modulate the host immune system. The efficacy of the host immune system can be impaired by *Staph. aureus* superantigen toxins (Wang et al., 2009), protein A, the polysaccharide capsule, biofilm formation, and the invasion/persistence strategy within host phagocytes as well as nonprofessional phagocytic cells (Middleton, 2008). More particularly, resistance against the action of circulating neutrophils and macrophages, which are the main actors in the immunity in the mammary gland, is believed to be an

important factor in the pathogenicity of *Staph. aureus*. Indeed, *Staph. aureus* has the ability to directly counteract opsonization, phagocytosis, and cytotoxic killing. Of particular interest is the leukocidin LukMF', a bi-component toxin specifically found in clonal lineages of *Staph. aureus* that are associated with bovine mastitis (Vrieling et al., 2015). The LukMF' leukocidin has an AA sequence closely related to that of the human-associated Panton-Valentine leukocidin (PVL; LukS-PV) and LukED and was shown to kill bovine migrating neutrophils through recognition of the CCR1 cell surface receptor with very high specificity and efficacy. It was also established that LukMF' is expressed in vivo during mastitis, although strain-to-strain disparity in expression levels has been reported (Vrieling et al., 2016). In the same study, the severity of mastitis clinical symptoms in experimentally infected cows was correlated with the in vivo expression level of LukMF' in milk. Likewise, a very recent genotypic and phenotypic analysis of clinical isolates from farms in the Netherlands found that *Staph. aureus* strains producing both high and low levels of LukMF' can be found in mastitis samples, of which 22% of the leukocidin-positive *Staph. aureus* isolates displayed a 10-fold higher LukMF' production than the average of the low-producing strains (Hoekstra et al., 2018). These high-producing isolates were cultured significantly more frequently from clinical than subclinical mastitis cases and all belonged to the same *Staphylococcus aureus* protein A (*spa*) type (t543).

DIVERSITY OF STAPH. AUREUS STRAINS THAT CAUSE MASTITIS

Staph. aureus Lineages

The immense diversity of strains capable of causing mastitis is a major obstacle in the development of an effective vaccine. Further disparity is present in the phenotypic aspects and the genomic presence of virulence genes among strains capable of causing the disease (Veh et al., 2015). In addition, the level of expression or the subset of *Staph. aureus* genes expressed during infection is profoundly different than that seen in vitro (Allard et al., 2006, 2013) or between strains (Capra et al., 2017). However, despite these discrepancies, the genomic constitution and phenotypic traits of *Staph. aureus* mastitis isolates are the result of a long-term evolutionary adaptation to the bovine host (Bergonier et al., 2014). Notwithstanding, such a host adaptation is very apparent when the different lineages of mastitis-causing strains are compared with human strains (Fitzgerald, 2012; Rainard et al., 2018). Outbreaks of

non-bovine-associated strains have been reported, some of which were caused by strains carrying antibiotic resistance cassettes (Hata, 2016).

Numerous molecular typing methods have been developed for epidemiological studies, including multi-locus sequence typing, pulsed-field gel electrophoresis, variable numbers of tandem repeat loci typing (Sabat et al., 2003; Pourcel et al., 2009), and analysis of the polymorphic X region of the protein A gene (*spa*). These typing methods can then be used to associate some strain types with certain genetic and phenotypic characteristics of *Staph. aureus* such as biofilm production, antimicrobial susceptibility, presence of specific virulence genes, and the ability to invade and persist within host cells (Veh et al., 2015; Ster et al., 2017). For instance, *S. aureus* mastitis strains of different sequence types were shown to produce a diversity of superantigen arrays, each having the ability to induce V β -specific proliferation of bovine T cells and with some arrays able to stimulate the entire T cell repertoire (Wilson et al., 2018). At the diagnostic level, an interesting approach lies in the potential use of molecular typing to predict the severity and persistence of IMI following identification of the strain causing infection. However, lineage may not always be the best marker to predict the severity of infection. Indeed, there could be important differences in the expression levels of toxins and proteases among genetically related *Staph. aureus* strains (Le Maréchal et al., 2011) or between in vitro and in vivo gene expression profiles (Allard et al., 2006). Furthermore, within a single strain, gene expression can change as the infection progresses over time (Jenkins et al., 2015).

Staph. aureus Small-Colony Variants

Over the last decades, a phenotypic variant of *Staph. aureus* has been isolated and characterized in many cases of chronic disease: the small-colony variant (SCV). As the name suggests, the main phenotypic feature associated with these subpopulations of isolates is slower growth, leading to the formation of small, non-pigmented and nonhemolytic colonies about one-tenth of the normal size of *Staph. aureus* colonies on solid culture medium (Proctor et al., 2006). These characteristics can be explained by the particular metabolic profile of SCV (Kriegeskorte et al., 2014). The majority of clinical *Staph. aureus* SCV are thought to derive from specific point mutations that lead to deficiencies in the electron transport chain, altering the oxidative phosphorylation process (Kahl et al., 2016).

In addition to the tolerance and resistance of SCV to certain antibiotics, which can be explained by

their slow growth and altered metabolism, a specific transcriptional signature allows expression of a set of virulence genes associated with persistence in the host. Small-colony variants have increased capacity for biofilm production (Mitchell et al., 2010; Singh et al., 2010) and an improved ability to invade and survive within host cells compared with prototypic *Staph. aureus* (Kalinka et al., 2014; Löffler et al., 2014). As mentioned above, the expression of virulence factors associated with dissemination and tissue invasion in prototypic *Staph. aureus* is, for the most part, under the control of the quorum-sensing mechanism (the Agr system). On the other hand, because of the slow growth of SCV, SigB preferentially influences the expression of adhesins and biofilm formation in SCV (Mitchell et al., 2013). It is generally accepted that SCV have increased ability to adhere and invade host cells due to high expression of FnBP and, once internalized in host cells, the intracellular persistence of SCV is due, at least in part, to decreased expression of α -hemolysin compared with prototypic *Staph. aureus* (Tuchscher et al., 2011). Interestingly, although the modified expression of FnBP and α -hemolysin is in fact attributable to the opposing influence of SigB and Agr, Agr could not promote the expression of α -hemolysin in absence of a functional transport chain, as is generally the case for SCV (Pader et al., 2014). Upon selective pressure (e.g., antibiotic treatment, the host immune response, or the intracellular milieu), SCV can be generated and, after release of the selective pressure, the prototypic phenotype can occur from the reversed mutation and be selected by its growth performance and dissemination abilities over the SCV phenotype. It was proposed that such a phenotypic switching is intrinsic to *Staph. aureus* pathogenesis and allows establishment of chronic infections (Tuchscher et al., 2011).

Small-colony variants are indeed frequently found in chronic infections (Kahl et al., 2016). In recent years, SCV have often been isolated from clinical samples of difficult-to-treat infections such as respiratory tract infections in cystic fibrosis patients, deep post-surgery infections, chronic osteomyelitis, and foreign body infections such as those associated with prosthetic joint and implants as well as from chronic cases of bovine mastitis (Atalla et al., 2008; Alkasir et al., 2013; Yagci et al., 2013; Kalinka et al., 2014; Yang et al., 2018). Certain factors present in these environments may favor the emergence of the persistent phenotype, including antibiotic treatments. Several studies have already reported resistance of SCV to many antibiotics (Vaudaux et al., 2011; Gläser et al., 2014), including aminoglycosides and some cationic peptides that depend on the respiratory chain and membrane potential ($\Delta\Psi$) for their mode

of action (Proctor et al., 2006). Also, the intracellular environment itself may contribute to the emergence of SCV (Tan et al., 2014). Unfortunately, it appears that the prevalence of SCV in the clinical context is still greatly undervalued, in part due to the difficulty in detecting the atypical *Staph. aureus* phenotype in clinical samples (Atalla et al., 2010; Yagci et al., 2013). Recurrent antibiotic treatments and internalization of *Staph. aureus* in epithelial mammary gland cells may indeed represent conditions proper to the generation of the SCV phenotype (Atalla et al., 2010) and potentially explain some of the relatively low cure rates observed for *Staph. aureus* IMI, which often require extended antibiotic therapies (Roy and Keefe, 2012). Furthermore, the host innate immune response for SCV is reduced compared with that observed for the normal phenotype (Ou et al., 2016), and SCV resist the oxidative burst of purified neutrophils and in blood (Painter et al., 2017). Together with the ability of SCV to invade and survive in host cells and their propensity to dynamically revert to an invasive phenotype under more permissive conditions, *Staph. aureus* survival strategies may thus contribute to the establishment of chronic infections by escaping the action of both antimicrobial therapies and host immunity.

VACCINE STRATEGIES

Targeting Bacterial Antigens Expressed During Infection

As mentioned above, *Staph. aureus* strains responsible for bovine mastitis are diverse and can be phenotypically very different. Moreover, *Staph. aureus* gene expression will vary considerably with the phase of infection and in response to the extracellular and intracellular environments. Consequently, a common protective antigen that would be useful against multiple *Staph. aureus* strains has not yet been discovered; very few subunit vaccine compositions have demonstrated substantial benefit to date (Middleton et al., 2009). Also, the selected antigen targets are not necessarily expressed in vivo by all strains or in the mammary gland environment. The in vivo environment can indeed modulate gene expression in *Staph. aureus* (Lowe et al., 1998; Allard et al., 2006). The in vivo expression of some specific known virulence factors was studied previously but it represented a limited selection of targets for vaccine development (Tollersrud et al., 2006). Other studies looked more broadly at *Staph. aureus* proteins and genes that could be expressed in vivo using in vitro culture conditions that could represent the mammary gland or milk environment (Lammers et al., 2000; Taverna et al., 2007).

Because these alternative media and growth conditions do not completely replicate the mammary gland environment or mastitic milk, our research team proceeded to experimentally infect cows to collect milk and bacteria during mastitis, and by a transcriptomic approach, examined genes that were strongly expressed by *Staph. aureus* during infection (Allard et al., 2013). Furthermore, in those experiments, several cows and multiple *Staph. aureus* strains of a variety of *spa* types were used to include host effects as well as strain genotypic and phenotypic diversity. Intramammary infections were followed for 18 d, which allowed us to identify a selection of bacterial genes that were strongly expressed by several *Staph. aureus* strains in a sustained manner during the infection period and in several cows. This approach led to identification of genes and protein candidates for vaccine or drug development.

Among others, gene *vraG* (coding for a putative ABC transporter permease) was shown to be strongly induced by *Staph. aureus* during mastitis (Allard et al., 2013). Because inactivation of that gene by mutagenesis led to a mutant that was significantly attenuated during experimental bovine IMI, the importance of *VraG* in virulence could be demonstrated (Allard et al., 2013). In fact, *VraG* seems to be required for cationic antimicrobial peptide sensing and resistance in *Staph. aureus* (Falord et al., 2012). Antimicrobial peptides are an important line of defense for the host against microbial pathogens (Hancock, 2001).

Examining *Staph. aureus* genes that are expressed during bovine mastitis also helped us identify novel drug targets, and we reported that the expression of the essential gene *guaA*, which codes for the enzyme guanosine monophosphate (GMP) synthase and is regulated by a guanine riboswitch (Mulhbachter et al., 2010), could be suppressed by a drug-like small-molecule riboswitch ligand acting as an “antibioswitch.” Such a novel class of antibiotic was shown to have some degree of success for the treatment of bovine mastitis (Ster et al., 2013).

In a recent study, an immunoproteomics approach was used to identify antigenic proteins from the surface of *Staph. aureus* to find new potential vaccine candidates (Misra et al., 2018). More specifically, proteins expressed by bacteria grown under iron-restricted conditions to mimic the mammalian host environment were extracted, separated, and blotted followed by specific detection with antibodies from mastitic milk. Thirty-eight *Staph. aureus* proteins were commonly detected by antibodies present in sera of several cows, of which 8 were predicted to be surface-associated and previously determined to be involved in *Staph. aureus* virulence. Of those, 2 surface proteins, the iron-regulated surface determinant protein C (IsdC) and ESAT-6 secretion

system extracellular protein EsxA, were cloned and purified in *E. coli* and then confirmed to be immunoreactive with antibodies from different cows with *Staph. aureus* mastitis. Misra et al. (2018) recognized, however, that this immunoproteomics approach could not distinguish between proteins that are reactive but not protective, although it could represent another effective way to identify new vaccine candidates.

Inactivated and Live Attenuated Vaccines

An inactivated *Staph. aureus* vaccine (StartVac or TopVac; Laboratorios Hipra S.A., Amer, Spain) is commercially available for bovine mastitis. This multivalent vaccine has a *Staph. aureus* component—a bacterin representing strains that express slime-associated antigens part of the biofilm extracellular matrix (Prenafeta et al., 2010). Although the use of a whole bacterin provides a selection of antigens that are suitable for raising an immune response, the success of such an approach is highly dependent on the diversity and type of the *Staph. aureus* strains present in herds. As such, discrepancies in the observed benefit of this vaccine reported in various studies may be attributed to the diversity of *Staph. aureus* strains, which varies geographically, as well as in the degree of slime and biofilm production that may depend on the bacterial growth phase or the environment (Schukken et al., 2014; Bradley et al., 2015; Landin et al., 2015).

Live-attenuated vaccines are still among the most widely used vaccination technologies today. Attenuated vaccines consist of bacterial or viral strains that are deliberately weakened until they are considered harmless or sufficiently less virulent, allowing them to only colonize the target host transiently. Traditionally, live attenuated vaccines have been developed by passage of pathogens under in vitro conditions until they lose their virulence. This empirical approach has been used in the case of the *Mycobacterium bovis* strain BCG for vaccination of humans against tuberculosis. It is now widely recommended that an attenuation strategy be more specific and precise; for example, by using targeted mutations affecting both virulence factors and metabolism (Galen and Curtiss, 2014). Complete gene deletions, compared with mutagenesis of single sites, are considered safer because of the impossibility of reversion, although the acquisition of complete functional virulence genes is theoretically possible via horizontal transfer from environmental bacteria (Frey, 2007).

Live vaccines have several advantages over formulations that use inactivated strains or subunit vaccines that contain purified antigens; for example, (1) they mimic a natural infection and therefore can cause im-

mune responses that are specific, localized, effective, and long lasting (Detmer and Glenting, 2006), and (2) they can prevent the first stages of infection by the pathogen, not just the symptoms of the disease (Frey, 2007). Live-attenuated vaccines also have the significant advantage of offering a broad range of antigenic targets and act intrinsically as what could be called natural adjuvants, by enhancing antigenic presentation through the stimulation of pattern recognition receptors (Griffiths and Khader, 2014). In addition, they can be used as antigenic live vectors for in situ expression of heterologous or modified antigenic proteins via plasmid or chromosomal expression systems. Many intracellular pathogens, such as *Listeria monocytogenes*, have not only been used for their live-attenuated vaccine qualities, but also for their ability to escape the phagolysosome after phagocytosis and to reach the cytoplasm of the infected host cells, where antigen presentation through the major histocompatibility complex (MHC)-I for stimulation of cellular immunity is possible (Bruhn et al., 2007). Live-attenuated *Salmonella typhimurium* (i.e., *Salmonella enterica* ssp. *enterica* serovar Typhimurium) strains were also used to deliver recombinant foreign antigens with interesting results (Zheng et al., 2012), notably with the combination of SPI-1 Type 3 secretion system and *Staph. aureus* recombinant EsxA and B proteins. Indeed, this approach allows the recognition of the antigens produced by the bacterium by both MHC-I and MHC-II, because the antigens are delivered by *Salmonella* directly into the cytoplasm.

We recently used a combination of the aforementioned elements to create a novel attenuated mutant of *Staph. aureus* that could be used as a vaccine for bovine mastitis (Côté-Gravel et al., 2016). The strategy took into consideration a critical phenotype part of *Staph. aureus* pathogenesis—SCV formation, which provides the possibility of delivering antigens in the host cell cytoplasmic compartment. Moreover, to avoid reversion to the invasive prototypic phenotype, we used a deletion of the *hemB* gene to create a stable and irreversible SCV phenotype. In addition, as seen in the *Staph. aureus* Small-Colony Variants subsection, although SCV are naturally attenuated in their virulence compared with prototypic strains, their ability to be internalized and to persist within host cells is an important aspect of *Staph. aureus* pathogenesis. Hence, a *vraG* mutation was introduced in the *hemB* SCV background to create an SCV double mutant incapable of sustaining an infection in a mouse mastitis model (Côté-Gravel et al., 2016). Indeed, as described in the Targeting Bacterial Antigens Expressed During Infection subsection, *vraG* is one of the *Staph. aureus* genes that is strongly

expressed during bovine mastitis and that was shown to be an important determinant of virulence in experimental bovine IMI (Allard et al., 2013). As a result, immunization of mice with such a live-attenuated double mutant raised a strong humoral response and antibodies that could also recognize strains from a variety of common mastitis associated *spa* types (Côté-Gravel et al., 2016). Additionally, significantly higher IgG_{2a}:IgG₁ titer ratios, which are indicative of a balanced T-helper (Th)1- and Th2-oriented immune response, were detected in sera obtained from mice immunized with the live-attenuated double mutant compared with those of mice vaccinated with the purified IsdH protein.

Immune Responses for Protection

The ability to stimulate a balanced humoral and cellular immune response may be key to achieving protection against *Staph. aureus*. Immunological response to *Staph. aureus* in the mammary gland of ruminants has been reviewed elsewhere (Hughes and Watson, 2018; Rainard et al., 2018). To summarize, the mammary gland of ruminants exploits various types of mechanisms to block the penetration and dissemination of microorganisms. As seen in the Pathogenesis of *Staph. aureus* IMI section, the gland is first protected against the entry of pathogens by structural defenses, among which the physical barrier constituted by the teat canal is undoubtedly one of the most important. The smooth muscle sphincters surrounding the teat canal also prevent leakage of milk by maintaining a tight closure and by producing a keratin layer by the epithelium lining of the duct between milking periods (Aitken et al., 2011). During lactation, the pressure build-up in the udder and the periodic opening of this canal during milking facilitate access of bacteria to the rest of the gland, where they are more likely to proliferate and cause an infection. Once bacteria have entered the cistern, the host innate immunity and adaptive immunity take over and operate in a coordinated manner. This specific response takes action with the presentation of pathogen antigens by specialized antigen-presenting cells (e.g., dendritic cells) and their recognition by naïve circulating B and T lymphocytes. The antigen-specific activated T and B cells then undergo clonal expansion of effector and memory cells through the triggering of specific proliferation cytokines that will influence the type of resulting lymphocyte subsets depending on the type of pathogen, MHC presentation pathway, and influence of cellular regulators during activation (Guzman et al., 2014). These subsets will then correspondingly influence the type of adaptive response that will occur in the gland by regulating specific and nonspecific im-

mune responses (Ezzat Alnakip et al., 2014). During the early stages of infection, the principal actions of innate immunity are unfolded through the pattern recognition receptor pathways and the subsequent outburst of pro-inflammatory cytokines, which include tumor necrosis factor α (TNF- α) and IL-1 β , among others (Oviedo-Boyso et al., 2007). These specific cytokines promote the rapid recruitment of specialized cellular actors such as the neutrophils and macrophages, which can directly ingest microorganisms by phagocytosis and produce soluble defense components; namely, myeloperoxidase and its generation of reactive oxygen species, antimicrobial peptides, immune-modulating proteins, oligosaccharides, lysozyme, lactoferrin, and complement system components (Aitken et al., 2011; Ezzat Alnakip et al., 2014). Neutrophils are the most important phagocytic cells and are the dominant innate cellular defense against mastitis-causing pathogens in the udder (Rainard and Riollet, 2006), and this seems specifically true against *Staph. aureus* (Spaan et al., 2013). When the innate immunity is not sufficient to clear pathogens, the host adaptive system is activated, and the dominance of neutrophils is shifted to an increasing number of lymphocytes and monocytes (Oviedo-Boyso et al., 2007).

The type of pathogen greatly influences the host response to infection (Petzl et al., 2018). Most coliform species that cause mastitis elicit a marked acute inflammatory response compared with *Staph. aureus* because of the presence of lipopolysaccharide (Ezzat Alnakip et al., 2014). Indeed, the outcome of a coliform-caused clinical mastitis is completely different from one originating from a persistent subclinical *Staph. aureus* IMI (Jensen et al., 2013). In a recent transcriptional analysis of the mammary gland's early response to an experimentally induced bovine mastitis, it was found that *Staph. aureus* IMI, in contrast to those caused by *E. coli*, did not modulate the expression level of any of the nuclear factor (NF)- κ B factors or their inhibitory I κ B regulators (Günther et al., 2017), as previously reported using *Staph. aureus*-stimulated mammary epithelial cells in vitro (Günther et al., 2011). Moreover, activation of the Wnt/ β -catenin cascade that provokes suppression of NF- κ B signaling and rearrangement of the actin cytoskeleton facilitate invasion of bacteria into host cells. Hence, Günther et al. (2017) proposed that *Staph. aureus* triggers some immune suppression during mastitis. This immunomodulation or dampening of immunity, together with the ability of *Staph. aureus* to invade and persist in host cells, can allow *Staph. aureus* to maintain an effective infection for an extended period. Similarly, another recent study revealed significant transcriptomic differences in microRNA expression of

bovine mammary glands infected with *Staph. aureus* and *E. coli* (Luoreng et al., 2018).

It is suggested that a vaccine that allows the development of a more balanced immunity, with a strong component of cell-mediated immunity, is necessary for protection against pathogens responsible for chronic infections (Kovacs-Nolan et al., 2009). More specifically, it appears that cell-mediated immunity based on Th1 and Th17 type responses may be necessary for protection (Lin et al., 2009; Spellberg and Daum, 2012; Fowler and Proctor, 2014). Thus, new ways to specifically stimulate the Th-1 and Th-17 pathways for the development of effective *Staph. aureus* vaccines are presently being explored (Lacey et al., 2017). It has been shown that antigen-specific Th-1 and Th-17 inflammatory responses are possible following intramammary immunization of cows with a sensitizing protein (Rainard et al., 2013). Another important goal would be to effectively increase the concentration of local or intramammary cell-mediated actors and antibodies in milk. For this purpose, different routes of immunization for vaccine administration, such as intramammary or intranasal inoculations, may provide ways to modulate and accentuate a local response (Boerhout et al., 2015).

ADJUVANTS AND IMMUNOMODULATION STRATEGIES

The development of a subunit vaccine that uses recombinant proteins was stimulated by several advantages, including the cost-effective preparation of specific and relevant antigens, thus eliminating host immune responses toward less important targets or suppressive antigens. Such an approach has, however, prompted a quest for adjuvants that could enhance both humoral and cellular immune responses. The diversity of adjuvants used in different vaccine formulations has been extensively reviewed by several authors (Nicholls et al., 2010; Savelkoul et al., 2015; Burakova et al., 2018). In addition, it has been shown recently that not only the type of adjuvant used for immunization but also the location of a subcutaneous injection can influence the isotypes and titers of antibodies produced in cattle; higher titers were effectively obtained from a prime immunization near the udder with an alum-saponin-oil adjuvant compared with immunization in the neck (Boerhout et al., 2018). The authors of that study hypothesized that with an immunization near the teat, antigens could be taken up by the supramammary lymph nodes, which are more likely to have previously been in natural contact with *Staph. aureus* compared with the prescapular lymph nodes in the neck, and therefore only a single dose of immunization was needed to induce similar effects.

Typical adjuvants include aluminum salts and oil-and-water emulsions of various types that can modulate the release of antigens. Others include pathogen-associated molecular patterns (PAMP) and toll-like receptor agonists that stimulate the innate immune response, as well as some polymers that can either carry antigens or act as immunostimulants on their own. Chitosan is one such a polymer that shows interesting properties. In a recent study, a chitosan-based formulation injected into the teat canal was shown to promote immune cell migration and accelerate mammary gland involution at dry-off, and it was proposed as an alternative to dry cow therapy (Lancôt et al., 2017). Chitosan is composed of D-glucosamine and N-acetyl-D-glucosamine units linked by β -1,4-glycosidic linkages, and its biological activity depends on its molecular weight and the degree of deacetylation. Interestingly, our group demonstrated that a 2.6-kDa chitosan (98% N-deacetylated) possessed antibacterial activity against *Staph. aureus* and could help reduce antibiotic use in dairy farms (Asli et al., 2017).

Approaches attempting direct immunomodulation of the udder by cytokines and other response modulators could represent alternatives to vaccination; such studies were reviewed by Petzl et al. (2018). A commercially available example of such an immunomodulator is the granulocyte colony stimulating factor pegbovigrastim (Imrestor; Elanco Animal Health, Greenfield, IN) that is proposed to help restoring neutrophil function during the periparturient period; that is, when cows are most susceptible to mastitis (Canning et al., 2017).

Finally, based on our own experience with live-attenuated strains as vaccines, which induce strong and balanced B and T cell immune responses compared with purified protein antigens, the concept of using microbe-mimicking or microbe-derived membrane vesicles for antigen delivery represents an attractive new perspective. Indeed, naturally occurring microbial membrane vesicles are increasingly recognized for their immunomodulatory effects on host immune responses (Kaparakis-Liaskos and Ferrero, 2015; Ahmadi Badi et al., 2017; Bitto and Kaparakis-Liaskos, 2017), and engineered-membrane vesicles can be used as vaccine delivery systems (Bai et al., 2011).

CONCLUSIONS

Bovine mastitis is the most common reason for antibiotic use in dairy farms. In the One Health concept, which acknowledges the interconnection between human and animal health and the environment, vaccines play a key role in disease prevention and are part of the effort to reduce the use of antibiotics. The challenges in the development of a vaccine for prevention

of *Staph. aureus* mastitis are several. In this review, we underscored the complex pathogenesis associated with this microorganism, which presents invasive and persistent phenotypes. Added to this is a large diversity of mastitis-causing strains, which reduces the probability of finding a universal vaccine. However, selection of relevant antigenic targets for vaccine development has been greatly aided by omics technologies but the need to stimulate both humoral and cellular immunity responses in order to control this bacterium requires innovative adjuvant or immunomodulatory strategies. Overall, a vaccine that offers full protection against *Staph. aureus* IMI is perhaps idealistic at this time but current and future approaches that could reduce disease prevalence should be viewed as important contributions in the field because of their direct effects on antibiotic use in dairy farms.

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REFERENCES

- Aghamohammadi, M., D. Haine, D. F. Kelton, H. W. Barkema, H. Hogeveen, G. P. Keefe, and S. Dufour. 2018. Herd-level mastitis-associated costs on Canadian dairy farms. *Front. Vet. Sci.* 5:100. <https://doi.org/10.3389/fvets.2018.00100>.
- Ahmadi Badi, S., A. Moshiri, A. Fateh, F. R. Jamnani, M. Sarshar, F. Vaziri, and S. D. Siadat. 2017. Microbiota-derived extracellular vesicles as new systemic regulators. *Front. Microbiol.* 8:1610. <https://doi.org/10.3389/fmicb.2017.01610>.
- Aitken, S. L., C. M. Corl, and L. M. Sordillo. 2011. Immunopathology of mastitis: Insights into disease recognition and resolution. *J. Mammary Gland Biol. Neoplasia* 16:291–304. <https://doi.org/10.1007/s10911-011-9230-4>.
- Alkasir, R., X. Liu, M. Zahra, M. Ferreri, J. Su, and B. Han. 2013. Characteristics of *Staphylococcus aureus* small colony variant and its parent strain isolated from chronic mastitis at a dairy farm in Beijing, China. *Microb. Drug Resist.* 19:138–145. <https://doi.org/10.1089/mdr.2012.0086>.
- Allard, M., H. Moisan, É. Brouillette, A. L. Gervais, M. Jacques, P. Lacasse, M. S. Diarra, and F. Malouin. 2006. Transcriptional modulation of some *Staphylococcus aureus* iron-regulated genes during growth in vitro and in a tissue cage model in vivo. *Microbes Infect.* 8:1679–1690. <https://doi.org/10.1016/j.micinf.2006.01.022>.
- Allard, M., C. Ster, C. L. Jacob, D. Scholl, M. S. Diarra, P. Lacasse, and F. Malouin. 2013. The expression of a putative exotoxin and an ABC transporter during bovine intramammary infection contributes to the virulence of *Staphylococcus aureus*. *Vet. Microbiol.* 162:761–770. <https://doi.org/10.1016/j.vetmic.2012.09.029>.
- Asli, A., E. Brouillette, C. Ster, M. G. Ghinet, R. Brzezinski, P. Lacasse, M. Jacques, and F. Malouin. 2017. Antibiofilm and antibacterial effects of specific chitosan molecules on *Staphylococcus aureus* isolates associated with bovine mastitis. *PLoS One* 12:e0176988. <https://doi.org/10.1371/journal.pone.0176988>.
- Atalla, H., C. Gyles, C. L. Jacob, H. Moisan, F. Malouin, and B. Mallard. 2008. Characterization of a *Staphylococcus aureus* small colony variant (SCV) associated with persistent bovine mastitis. *Foodborne Pathog. Dis.* 5:785–799. <https://doi.org/10.1089/fpd.2008.0110>.
- Atalla, H., C. Gyles, and B. Mallard. 2010. Persistence of a *Staphylococcus aureus* small colony variants (*S. aureus* SCV) within bovine mammary epithelial cells. *Vet. Microbiol.* 143:319–328. <https://doi.org/10.1016/j.vetmic.2009.11.030>.
- Bai, X., J. Findlow, and R. Borrow. 2011. Recombinant protein meningococcal serogroup B vaccine combined with outer membrane vesicles. *Expert Opin. Biol. Ther.* 11:969–985. <https://doi.org/10.1517/14712598.2011.585965>.
- Bar, D., L. W. Tauer, G. Bennett, R. N. González, J. A. Hertl, Y. H. Schukken, H. F. Schulte, F. L. Welcome, and Y. T. Gröhn. 2008. The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. *J. Dairy Sci.* 91:2205–2214. <https://doi.org/10.3168/jds.2007-0573>.
- Barkema, H. W., Y. H. Schukken, and R. N. Zadoks. 2006. Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. *J. Dairy Sci.* 89:1877–1895. [https://doi.org/10.3168/jds.S0022-0302\(06\)72256-1](https://doi.org/10.3168/jds.S0022-0302(06)72256-1).
- Bergonier, D., D. Sobral, A. T. Feßler, E. Jacquet, F. B. Gilbert, S. Schwarz, M. Treilles, P. Bouloc, C. Pourcel, and G. Vergnaud. 2014. *Staphylococcus aureus* from 152 cases of bovine, ovine and caprine mastitis investigated by Multiple-locus variable number of tandem repeat analysis (MLVA). *Vet. Res.* 45:97. <https://doi.org/10.1186/s13567-014-0097-4>.
- Bitto, N. J., and M. Kaparakis-Liaskos. 2017. The therapeutic benefit of bacterial membrane vesicles. *Int. J. Mol. Sci.* 18:1287. <https://doi.org/10.3390/ijms18061287>.
- Boerhout, E., M. Vrieling, L. Benedictus, I. Daemen, L. Ravesloot, V. Rutten, P. Nuijten, J. van Strijp, A. Koets, and S. Eisenberg. 2015. Immunization routes in cattle impact the levels and neutralizing capacity of antibodies induced against *S. aureus* immune evasion proteins. *Vet. Res.* 46:115. <https://doi.org/10.1186/s13567-015-0243-7>.
- Boerhout, E. M., A. P. Koets, T. G. T. Mols-Vorstermans, P. J. M. Nuijten, M. J. H. Hoeijmakers, V. P. M. G. Rutten, and J. J. E. Bijlsma. 2018. The antibody response in the bovine mammary gland is influenced by the adjuvant and the site of subcutaneous vaccination. *Vet. Res.* 49:25. <https://doi.org/10.1186/s13567-018-0521-2>.
- Boisset, S., T. Geissmann, E. Huntzinger, P. Fechter, N. Bendridi, M. Possedko, C. Chevalier, A. C. Helfer, Y. Benito, A. Jacquier, C. Gaspin, F. Vandenesch, and P. Romby. 2007. *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. *Genes Dev.* 21:1353–1366. <https://doi.org/10.1101/gad.423507>.
- Bradley, A. J., J. E. Breen, B. Payne, V. White, and M. J. Green. 2015. An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom. *J. Dairy Sci.* 98:1706–1720. <https://doi.org/10.3168/jds.2014-8332>.

- Brouillette, E., B. G. Talbot, and F. Malouin. 2003. The fibronectin-binding proteins of *Staphylococcus aureus* may promote mammary gland colonization in a lactating mouse model of mastitis. *Infect. Immun.* 71:2292–2295. <https://doi.org/10.1128/IAI.71.4.2292-2295.2003>.
- Bruhn, K. W., N. Craft, and J. Miller. 2007. *Listeria* as a vaccine vector. *Microbes Infect.* 9:1226–1235. <https://doi.org/10.1016/j.micinf.2007.05.010>.
- Burakova, Y., R. Madera, S. McVey, J. R. Schlup, and J. Shi. 2018. Adjuvants for animal vaccines. *Viral Immunol.* 31:11–22. <https://doi.org/10.1089/vim.2017.0049>.
- Canning, P., R. Hassfurth, T. TerHune, K. Rogers, S. Abbott, and D. Kolb. 2017. Efficacy and clinical safety of pegbovigrastim for preventing naturally occurring clinical mastitis in periparturient primiparous and multiparous cows on US commercial dairies. *J. Dairy Sci.* 100:6504–6515. <https://doi.org/10.3168/jds.2017-12583>.
- Capra, E., P. Cremonesi, A. Pietrelli, S. Puccio, M. Luini, A. Stella, and B. Castiglioni. 2017. Genomic and transcriptomic comparison between *Staphylococcus aureus* strains associated with high and low within herd prevalence of intra-mammary infection. *BMC Microbiol.* 17:21. <https://doi.org/10.1186/s12866-017-0931-8>.
- Condas, L. A. Z., J. De Buck, D. B. Nobrega, D. A. Carson, S. Naushad, S. De Vliegher, R. N. Zadoks, J. R. Middleton, S. Dufour, J. P. Kastelic, and H. W. Barkema. 2017. Prevalence of non-*aureus* staphylococci species causing intramammary infections in Canadian dairy herds. *J. Dairy Sci.* 100:5592–5612. <https://doi.org/10.3168/jds.2016-12478>.
- Contreras, G. A., and J. M. Rodriguez. 2011. Mastitis: Comparative etiology and epidemiology. *J. Mammary Gland Biol. Neoplasia* 16:339–356. <https://doi.org/10.1007/s10911-011-9234-0>.
- Côté-Gravel, J., E. Brouillette, N. Obradović, C. Ster, B. G. Talbot, and F. Malouin. 2016. Characterization of a *vraG* mutant in a genetically stable *Staphylococcus aureus* small-colony variant and preliminary assessment for use as a live-attenuated vaccine against intramammary infections. *PLoS One* 11:e0166621. <https://doi.org/10.1371/journal.pone.0166621>.
- Detmer, A., and J. Glenting. 2006. Live bacterial vaccines—A review and identification of potential hazards. *Microb. Cell Fact.* 5:23. <https://doi.org/10.1186/1475-2859-5-23>.
- Dinsmore, R. P. 2002. Biosecurity for mammary diseases in dairy cattle. *Vet. Clin. North Am. Food Anim. Pract.* 18:115–131.
- Ezzat Alnakip, M., M. Quintela-Baluja, K. Böhme, I. Fernández-No, S. Caamaño-Antelo, P. Calo-Mata, and J. Barros-Velázquez. 2014. The immunology of mammary gland of dairy ruminants between healthy and inflammatory conditions. *J. Vet. Med.* 2014:659801. <https://doi.org/10.1155/2014/659801>.
- Falord, M., G. Karimova, A. Hiron, and T. Msadek. 2012. GraXSR proteins interact with the *VraFG* ABC transporter to form a five-component system required for cationic antimicrobial peptide sensing and resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 56:1047–1058. <https://doi.org/10.1128/AAC.05054-11>.
- Fitzgerald, J. R. 2012. Livestock-associated *Staphylococcus aureus*: Origin, evolution and public health threat. *Trends Microbiol.* 20:192–198. <https://doi.org/10.1016/j.tim.2012.01.006>.
- Fowler, V. G., and R. Proctor. 2014. Where does a *Staphylococcus aureus* vaccine stand? *Clin. Microbiol. Infect.* 20(Suppl. 5):66–75. <https://doi.org/10.1111/1469-0691.12570>.
- Frey, J. 2007. Biological safety concepts of genetically modified live bacterial vaccines. *Vaccine* 25:5598–5605. <https://doi.org/10.1016/j.vaccine.2006.11.058>.
- Galen, J. E., and R. Curtiss. 2014. The delicate balance in genetically engineering live vaccines. *Vaccine* 32:4376–4385. <https://doi.org/10.1016/j.vaccine.2013.12.026>.
- García-Álvarez, L., M. T. G. Holden, H. Lindsay, C. R. Webb, D. F. J. Brown, M. D. Curran, E. Walpole, K. Brooks, D. J. Pickard, C. Teale, J. Parkhill, S. D. Bentley, G. F. Edwards, E. K. Girvan, A. M. Kearns, B. Pichon, R. L. R. Hill, A. R. Larsen, R. L. Skov, S. J. Peacock, D. J. Maskell, and M. A. Holmes. 2011. Methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: A descriptive study. *Lancet Infect. Dis.* 11:595–603. [https://doi.org/10.1016/S1473-3099\(11\)70126-8](https://doi.org/10.1016/S1473-3099(11)70126-8).
- Gläser, R., K. Becker, C. von Eiff, U. Meyer-Hoffert, and J. Harder. 2014. Decreased susceptibility of *Staphylococcus aureus* small-colony variants toward human antimicrobial peptides. *J. Invest. Dermatol.* 134:2347–2350. <https://doi.org/10.1038/jid.2014.176>.
- Griffiths, K. L., and S. A. Khader. 2014. Novel vaccine approaches for protection against intracellular pathogens. *Curr. Opin. Immunol.* 28:58–63. <https://doi.org/10.1016/j.coi.2014.02.003>.
- Günther, J., K. Esch, N. Poschadel, W. Petzl, H. Zerbe, S. Mitterhumer, H. Blum, and H. M. Seyfert. 2011. Comparative kinetics of *Escherichia coli*- and *Staphylococcus aureus*-specific activation of key immune pathways in mammary epithelial cells demonstrates that *S. aureus* elicits a delayed response dominated by interleukin-6 (IL-6) but not by IL-1A or tumor. *Infect. Immun.* 79:695–707. <https://doi.org/10.1128/IAI.01071-10>.
- Günther, J., W. Petzl, I. Bauer, S. Ponsuksili, H. Zerbe, H. J. Schuberth, R. M. Brunner, and H. M. Seyfert. 2017. Differentiating *Staphylococcus aureus* from *Escherichia coli* mastitis: *S. aureus* triggers unbalanced immune-dampening and host cell invasion immediately after udder infection. *Sci. Rep.* 7:4811. <https://doi.org/10.1038/s41598-017-05107-4>.
- Guzman, E., J. Hope, G. Taylor, A. L. Smith, C. Cubillos-Zapata, and B. Charleston. 2014. Bovine $\gamma\delta$ T cells are a major regulatory T cell subset. *J. Immunol.* 193:208–222. <https://doi.org/10.4049/jimmunol.1303398>.
- Halasa, T., K. Huijps, O. Østerås, and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: A review. *Vet. Q.* 29:18–31. <https://doi.org/10.1080/01652176.2007.9695224>.
- Hancock, R. E. 2001. Cationic peptides: Effectors in innate immunity and novel antimicrobials. *Lancet Infect. Dis.* 1:156–164. [https://doi.org/10.1016/S1473-3099\(01\)00092-5](https://doi.org/10.1016/S1473-3099(01)00092-5).
- Hata, E. 2016. Bovine mastitis outbreak in Japan caused by methicillin-resistant *Staphylococcus aureus* New York/Japan clone. *J. Vet. Diagn. Invest.* 28:291–298. <https://doi.org/10.1177/1040638716643126>.
- Hathroubi, S., M. A. Mekni, P. Domenico, D. Nguyen, and M. Jacques. 2017. Biofilms: Microbial Shelters Against Antibiotics. *Microb. Drug Resist.* 23:147–156. <https://doi.org/10.1089/mdr.2016.0087>.
- Hoekstra, J., V. Rutten, L. Sommeling, T. van Werven, M. Spaninks, B. Duim, L. Benedictus, and G. Koop. 2018. High production of LukMF in *Staphylococcus aureus* field strains is associated with clinical bovine mastitis. *Toxins (Basel)* 10:200. <https://doi.org/10.3390/toxins10050200>.
- Hogan, J., and K. L. Smith. 2003. Coliform mastitis. *Vet. Res.* 34:507–519. <https://doi.org/10.1051/vetres:2003022>.
- Holmes, M. A., and R. N. Zadoks. 2011. Methicillin resistant *S. aureus* in human and bovine mastitis. *J. Mammary Gland Biol. Neoplasia* 16:373–382. <https://doi.org/10.1007/s10911-011-9237-x>.
- Horsburgh, M. J., J. L. Aish, I. J. White, J. K. Lithgow, S. J. Foster, and L. Shaw. 2002. sigmaB modulates virulence determinant expression and stress resistance: Characterization of a functional *rsbU* strain derived from *Staphylococcus aureus* 8325-4. *J. Bacteriol.* 184:5457–5467. <https://doi.org/10.1128/JB.184.19.5457>.
- Hughes, K., and C. J. Watson. 2018. The mammary microenvironment in mastitis in humans, dairy ruminants, rabbits and rodents: A One Health focus. *J. Mammary Gland Biol. Neoplasia* 23:27–41. <https://doi.org/10.1007/s10911-018-9395-1>.
- Ismail, Z. B. 2017. Mastitis vaccines in dairy cows: Recent developments and recommendations of application. *Vet. World* 10:1057–1062. <https://doi.org/10.14202/vetworld.2017.1057-1062>.
- Jenkins, A., A. Diep, T. T. Mai, N. H. Vo, P. Warrenner, J. Suzich, C. K. Stover, and R. Sellman. 2015. Differential expression and roles of *Staphylococcus aureus* virulence determinants during colonization and disease. *MBio* 6:e02272–e14. <https://doi.org/10.1128/mBio.02272-14>.
- Jensen, K., J. Günther, R. Talbot, W. Petzl, H. Zerbe, H. J. Schuberth, H. M. Seyfert, and E. J. Glass. 2013. *Escherichia coli*- and *Staphylococcus aureus*-induced mastitis differentially modulate transcrip-

- tional responses in neighbouring uninfected bovine mammary gland quarters. *BMC Genomics* 14:36. <https://doi.org/10.1186/1471-2164-14-36>.
- Kahl, B. C., K. Becker, and B. Löffler. 2016. Clinical significance and pathogenesis of staphylococcal small colony variants in persistent infections. *Clin. Microbiol. Rev.* 29:401–427. <https://doi.org/10.1128/CMR.00069-15>.
- Kalinka, J., M. Hachmeister, J. Geraci, D. Sordelli, U. Hansen, S. Niemann, S. Oetermann, G. Peters, B. Löffler, and L. Tuchscher. 2014. *Staphylococcus aureus* isolates from chronic osteomyelitis are characterized by high host cell invasion and intracellular adaptation, but still induce inflammation. *Int. J. Med. Microbiol.* <https://doi.org/10.1016/j.ijmm.2014.07.013>.
- Kaparakis-Liaskos, M., and R. L. Ferrero. 2015. Immune modulation by bacterial outer membrane vesicles. *Nat. Rev. Immunol.* 15:375–387. <https://doi.org/10.1038/nri3837>.
- Kovacs-Nolan, J., J. W. Mapletoft, L. Latimer, L. A. Babiuk, and S. V. D. Littel-van den Hurk. 2009. CpG oligonucleotide, host defense peptide and polyphosphazene act synergistically, inducing long-lasting, balanced immune responses in cattle. *Vaccine* 27:2048–2054. <https://doi.org/10.1016/j.vaccine.2009.01.117>.
- Kriegeskorte, A., S. Grubmüller, C. Huber, B. C. Kahl, C. von Eiff, R. A. Proctor, G. Peters, W. Eisenreich, and K. Becker. 2014. *Staphylococcus aureus* small colony variants show common metabolic features in central metabolism irrespective of the underlying auxotrophism. *Front. Cell. Infect. Microbiol.* 4:141. <https://doi.org/10.3389/fcimb.2014.00141>.
- Lacey, K. A., J. M. Leech, S. J. Lalor, N. McCormack, J. A. Geoghegan, and R. M. McLoughlin. 2017. The *Staphylococcus aureus* cell wall-anchored protein clumping factor A is an important T cell antigen. *Infect. Immun.* 85:1–12. <https://doi.org/10.1128/IAI.00549-17>.
- Lammers, A., E. Kruijt, C. Van De Kuit, P. J. M. Nuijten, and H. E. Smith. 2000. Identification of *Staphylococcus aureus* genes expressed during growth in milk: A useful model for selection of genes important in bovine mastitis? *Microbiology* 146:981–987. <https://doi.org/10.1099/00221287-146-4-981>.
- Lancôt, S., P. Fustier, A. R. Taherian, B. Bisakowski, X. Zhao, and P. Lacasse. 2017. Effect of intramammary infusion of chitosan hydrogels at drying-off on bovine mammary gland involution. *J. Dairy Sci.* 100:2269–2281. <https://doi.org/10.3168/jds.2016-12087>.
- Landin, H., M. J. Mörk, M. Larsson, and K. P. Waller. 2015. Vaccination against *Staphylococcus aureus* mastitis in two Swedish dairy herds. *Acta Vet. Scand.* 57:81. <https://doi.org/10.1186/s13028-015-0171-6>.
- Le Maréchal, C., N. Seyffert, J. Jardin, D. Hernandez, G. Jan, L. Rault, V. Azevedo, P. François, J. Schrenzel, M. van de Guchte, S. Even, N. Berkova, R. Thiéry, J. R. Fitzgerald, E. Vauto, and Y. Le Loir. 2011. Molecular basis of virulence in *Staphylococcus aureus* mastitis. *PLoS One* 6:e27354. <https://doi.org/10.1371/journal.pone.0027354>.
- Li, L., and X. Zhao. 2018. Characterization of the resistance class 1 integrons in *Staphylococcus aureus* isolates from milk of lactating dairy cattle in Northwestern China. *BMC Vet. Res.* 14:59.
- Lin, L., A. S. Ibrahim, X. Xu, J. M. Farber, V. Avanesian, B. Baquir, Y. Fu, S. W. French, J. E. Edwards, and B. Spellberg. 2009. Th1-Th17 cells mediate protective adaptive immunity against *Staphylococcus aureus* and *Candida albicans* infection in mice. *PLoS Pathog.* 5:e1000703. <https://doi.org/10.1371/journal.ppat.1000703>.
- Löffler, B., L. Tuchscher, S. Niemann, and G. Peters. 2014. *Staphylococcus aureus* persistence in non-professional phagocytes. *Int. J. Med. Microbiol.* <https://doi.org/10.1016/j.ijmm.2013.11.011>.
- Lowe, A. M., D. T. Beattie, and R. L. Deresiewicz. 1998. Identification of novel staphylococcal virulence genes by *in vivo* expression technology. *Mol. Microbiol.* 27:967–976. <https://doi.org/10.1046/j.1365-2958.1998.00741.x>.
- Luoreng, Z. M., X. P. Wang, C. G. Mei, and L. Sen Zan. 2018. Comparison of microRNA profiles between bovine mammary glands infected with *Staphylococcus aureus* and *Escherichia coli*. *Int. J. Biol. Sci.* 14:87–99. <https://doi.org/10.7150/ijbs.22498>.
- Middleton, J. R. 2008. *Staphylococcus aureus* antigens and challenges in vaccine development for humans and dairy cattle. *Expert Rev. Vaccines* 7:1–11.
- Middleton, J. R., C. D. Luby, and D. S. Adams. 2009. Efficacy of vaccination against staphylococcal mastitis: A review and new data. *Vet. Microbiol.* 134:192–198. <https://doi.org/10.1016/j.vetmic.2008.09.053>.
- Misra, N., X. Pu, D. N. Holt, M. A. McGuire, and J. K. Tinker. 2018. Immunoproteomics to identify *Staphylococcus aureus* antigens expressed in bovine milk during mastitis. *J. Dairy Sci.* 101:6296–6309. <https://doi.org/10.3168/jds.2017-14040>.
- Mitchell, G., A. Fugère, K. Pépin Gaudreau, E. Brouillette, E. H. Frost, A. M. Cantin, and F. Malouin. 2013. SigB is a dominant regulator of virulence in *Staphylococcus aureus* small-colony variants. *PLoS One* 8:e65018. <https://doi.org/10.1371/journal.pone.0065018>.
- Mitchell, G., C.-A. Lamontagne, E. Brouillette, G. Grondin, B. G. Talbot, M. Grandbois, and F. Malouin. 2008. *Staphylococcus aureus* SigB activity promotes a strong fibronectin-bacterium interaction which may sustain host tissue colonization by small-colony variants isolated from cystic fibrosis patients. *Mol. Microbiol.* 70:1540–1555. <https://doi.org/10.1111/j.1365-2958.2008.06511.x>.
- Mitchell, G., D. L. Séguin, A. Asselin, E. Déziel, A. M. Cantin, E. H. Frost, S. Michaud, and F. Malouin. 2010. *Staphylococcus aureus* sigma B-dependent emergence of small-colony variants and biofilm production following exposure to *Pseudomonas aeruginosa* 4-hydroxy-2-heptylquinoline-N-oxide. *BMC Microbiol.* 10:33.
- Mulhbach, J., E. Brouillette, M. Allard, L.-C. Fortier, F. Malouin, and D. A. Lafontaine. 2010. Novel riboswitch ligand analogs as selective inhibitors of guanine-related metabolic pathways. *PLoS Pathog.* 6:e1000865. <https://doi.org/10.1371/journal.ppat.1000865>.
- Murai, K., T. W. Lehenbauer, J. D. Champagne, K. Glenn, and S. S. Aly. 2014. Cost-effectiveness of diagnostic strategies using quantitative real-time PCR and bacterial culture to identify contagious mastitis cases in large dairy herds. *Prev. Vet. Med.* 113:522–535. <https://doi.org/10.1016/j.prevetmed.2014.01.001>.
- Naqvi, S. A., J. De Buck, S. Dufour, and H. W. Barkema. 2018. Udder health in Canadian dairy heifers during early lactation. *J. Dairy Sci.* 101:3233–3247. <https://doi.org/10.3168/jds.2017-13579>.
- Nicholls, E. F., L. Madera, and R. E. W. Hancock. 2010. Immunomodulators as adjuvants for vaccines and antimicrobial therapy. *Ann. N. Y. Acad. Sci.* 1213:46–61. <https://doi.org/10.1111/j.1749-6632.2010.05787.x>.
- Novick, R. P., and E. Geisinger. 2008. Quorum sensing in staphylococci. *Annu. Rev. Genet.* 42:541–564. <https://doi.org/10.1146/annurev.genet.42.110807.091640>.
- Olde Riekerink, R. G. M., H. W. Barkema, D. F. Kelton, and D. T. Scholl. 2008. Incidence rate of clinical mastitis on Canadian dairy farms. *J. Dairy Sci.* 91:1366–1377. <https://doi.org/10.3168/jds.2007-0757>.
- Otto, M. 2013. Staphylococcal infections: Mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. *Annu. Rev. Med.* 64:175–188. <https://doi.org/10.1146/annurev-med-042711-140023>.
- Ou, J. J. J., A. J. Drilling, C. Cooksley, A. Bassiouni, S. P. Kidd, A. J. Psaltis, P. J. Wormald, and S. Vreugde. 2016. Reduced innate immune response to a *Staphylococcus aureus* small colony variant compared to its wild-type parent strain. *Front. Cell. Infect. Microbiol.* 6:187. <https://doi.org/10.3389/fcimb.2016.00187>.
- Oviedo-Boyso, J., J. J. Valdez-Alarcon, M. Cajero-Juarez, A. Ochoa-Zarzosa, J. E. Lopez-Meza, A. Bravo-Patino, and V. M. Baizabal-Aguirre. 2007. Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. *J. Infect.* 54:399–409. <https://doi.org/10.1016/j.jinf.2006.06.010>.
- Pader, V., E. H. James, K. L. Painter, S. Wigneshweraraj, and A. M. Edwards. 2014. The Agr quorum-sensing system regulates fibronectin-binding but not hemolysis in the absence of a functional electron transport chain. *Infect. Immun.* 82:4337–4347. <https://doi.org/10.1128/IAI.02254-14>.

- Painter, K. L., A. Hall, K. P. Ha, and A. M. Edwards. 2017. The electron transport chain sensitizes *Staphylococcus aureus* and *Enterococcus faecalis* to the oxidative burst. *Infect. Immun.* 85:1–13.
- Petrovski, K. R., M. Trajcev, and G. Buneski. 2006. A review of the factors affecting the costs of bovine mastitis. *J. S. Afr. Vet. Assoc.* 77:52–60.
- Petzl, W., H. Zerbe, J. Günther, H. M. Seyfert, J. Hussen, and H. J. Schubert. 2018. Pathogen-specific responses in the bovine udder. Models and immunoprophylactic concepts. *Res. Vet. Sci.* 116:55–61. <https://doi.org/10.1016/j.rvsc.2017.12.012>.
- Pourcel, C., K. Hormigos, L. Onteniente, O. Sakwinska, R. H. Deurenberg, and G. Vergnaud. 2009. Improved MLVA assay for *Staphylococcus aureus* providing a highly informative genotyping technique together with strong phylogenetic value. *J. Clin. Microbiol.* 47:3121–3128. <https://doi.org/10.1128/JCM.00267-09>.
- Prenafeta, A., R. March, A. Foix, I. Casals, and L. Costa. 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. *Vet. Immunol. Immunopathol.* 134:208–217. <https://doi.org/10.1016/j.vetimm.2009.09.020>.
- Proctor, R. A., C. von Eiff, B. C. Kahl, K. Becker, P. McNamara, M. Herrmann, and G. Peters. 2006. Small colony variants: A pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat. Rev. Microbiol.* 4:295–305. <https://doi.org/10.1038/nrmicro1384>.
- Rainard, P., P. Cunha, S. Bougarn, A. Fromageau, C. Rossignol, F. B. Gilbert, and P. Berthon. 2013. T helper 17-associated cytokines are produced during antigen-specific inflammation in the mammary gland. *PLoS One* 8:e63471. <https://doi.org/10.1371/journal.pone.0063471>.
- Rainard, P., G. Foucras, J. R. Fitzgerald, J. L. Watts, G. Koop, and J. R. Middleton. 2018. Knowledge gaps and research priorities in *Staphylococcus aureus* mastitis control. *Transbound. Emerg. Dis.* 65:149–165. <https://doi.org/10.1111/tbed.12698>.
- Rainard, P., and C. R. Riollet. 2006. Innate immunity of the bovine mammary gland. *Vet. Res.* 37:369–400. <https://doi.org/10.1051/vetres:2006007>.
- Reyher, K. K., S. Dufour, H. W. Barkema, L. Des Côteaux, T. J. Devries, I. R. Dohoo, G. P. Keefe, J.-P. Roy, and D. T. Scholl. 2011. The National Cohort of Dairy Farms—A data collection platform for mastitis research in Canada. *J. Dairy Sci.* 94:1616–1626. <https://doi.org/10.3168/jds.2010-3180>.
- Roy, J.-P., and G. Keefe. 2012. Systematic review: What is the best antibiotic treatment for *Staphylococcus aureus* intramammary infection of lactating cows in North America? *Vet. Clin. North Am. Food Anim. Pract.* 28:39–50. <https://doi.org/10.1016/j.cvfa.2011.12.004>.
- Ruegg, P. L. 2017. A 100-Year Review : Mastitis detection, management, and prevention. *J. Dairy Sci.* 100:10381–10397. <https://doi.org/10.3168/jds.2017-13023>.
- Sabat, A., J. Krzyszton-russjan, W. Strzalka, R. Filipek, K. Kosowska, W. Hryniewicz, J. Travis, and J. Potempa. 2003. New method for typing *Staphylococcus aureus* strains: Multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. *J. Clin. Microbiol.* 41:1801. <https://doi.org/10.1128/JCM.41.4.1801>.
- Savelkoul, H. F., V. Ferro, M. Strioga, and V. Schijns. 2015. Choice and design of adjuvants for parenteral and mucosal vaccines. *Vaccines (Basel)* 3:148–171. <https://doi.org/10.3390/vaccines3010148>.
- Schukken, Y. H., V. Bronzo, C. Locatelli, C. Pollera, N. Rota, A. Casula, F. Testa, L. Scaccabarozzi, R. March, D. Zalduendo, R. Guix, and P. Moroni. 2014. Efficacy of vaccination on *Staphylococcus aureus* and coagulase-negative staphylococci intramammary infection dynamics in 2 dairy herds. *J. Dairy Sci.* 97:5250–5264. <https://doi.org/10.3168/jds.2014-8008>.
- Shim, E. H., R. D. Shanks, and D. E. Morin. 2004. Milk loss and treatment costs associated with two treatment protocols for clinical mastitis in dairy cows. *J. Dairy Sci.* 87:2702–2708. [https://doi.org/10.3168/jds.S0022-0302\(04\)73397-4](https://doi.org/10.3168/jds.S0022-0302(04)73397-4).
- Singh, R., P. Ray, A. Das, and M. Sharma. 2010. Enhanced production of exopolysaccharide matrix and biofilm by a menadione-auxotrophic *Staphylococcus aureus* small-colony variant. *J. Med. Microbiol.* 59:521–527. <https://doi.org/10.1099/jmm.0.017046-0>.
- Spaan, A. N., B. G. J. Surewaard, R. Nijland, and J. A. G. van Strijp. 2013. Neutrophils versus *Staphylococcus aureus* : A biological tug of war. *Annu. Rev. Microbiol.* 67:629–650. <https://doi.org/10.1146/annurev-micro-092412-155746>.
- Spellberg, B., and R. Daum. 2012. Development of a vaccine against *Staphylococcus aureus*. *Semin. Immunopathol.* 34:335–348. <https://doi.org/10.1007/s00281-011-0293-5>.
- Ster, C., M. Allard, S. Boulanger, M. Lamontagne Boulet, J. Mulhbach, D. A. Lafontaine, E. Marsault, P. Lacasse, and F. Malouin. 2013. Experimental treatment of *Staphylococcus aureus* bovine intramammary infection using a guanine riboswitch ligand analog. *J. Dairy Sci.* 96:1000–1008. <https://doi.org/10.3168/jds.2012-5890>.
- Ster, C., V. Lebeau, J. Leclerc, A. Fugère, K. A. Veh, J. P. Roy, and F. Malouin. 2017. *In vitro* antibiotic susceptibility and biofilm production of *Staphylococcus aureus* isolates recovered from bovine intramammary infections that persisted or not following extended therapies with cephapirin, pirlimycin or ceftiofur. *Vet. Res.* 48:56. <https://doi.org/10.1186/s13567-017-0463-0>.
- Suriyaphol, G., M. Sarikaputi, and P. Suriyaphol. 2009. Differential responses of cells from human skin keratinocyte and bovine mammary epithelium to attack by pore-forming *Staphylococcus aureus* alpha-toxin. *Comp. Immunol. Microbiol. Infect. Dis.* 32:491–502. <https://doi.org/10.1016/j.cimid.2008.07.002>.
- Sutra, L., and B. Poutrel. 1994. Virulence factors involved in the pathogenesis of bovine intramammary infections due to *Staphylococcus aureus*. *J. Med. Microbiol.* 40:79–89.
- Tan, N. C. W., C. M. Cooksley, E. Roscioli, A. J. Drilling, R. Douglas, P. J. Wormald, and S. Vreugde. 2014. Small-colony variants and phenotype switching of intracellular *Staphylococcus aureus* in chronic rhinosinusitis. *Allergy* 69:1364–1371. <https://doi.org/10.1111/all.12457>.
- Taverna, F., A. Negri, R. Piccinini, A. Zecconi, S. Nonnis, S. Ronchi, and G. Tedeschi. 2007. Characterization of cell wall associated proteins of a *Staphylococcus aureus* isolated from bovine mastitis case by a proteomic approach. *Vet. Microbiol.* 119:240–247. <https://doi.org/10.1016/j.vetmic.2006.09.007>.
- Tollersrud, T., A. H. Kampen, and K. Kenny. 2006. *Staphylococcus aureus* enterotoxin D is secreted in milk and stimulates specific antibody responses in cows in the course of experimental intramammary infection. *Infect. Immun.* 74:3507–3512. <https://doi.org/10.1128/IAI.01726-05>.
- Tuchscher, L., E. Medina, M. Hussain, W. Völker, V. Heitmann, S. Niemann, D. Holzinger, J. Roth, R. A. Proctor, K. Becker, G. Peters, and B. Löffler. 2011. *Staphylococcus aureus* phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. *EMBO Mol. Med.* 3:129–141. <https://doi.org/10.1002/emmm.201000115>.
- Vaudaux, P., E. Huggler, W. Rhys-Williams, W. G. Love, and D. P. Lew. 2011. Extracellular and intracellular bactericidal activities of XF-70 against small-colony variant *hemB* mutants of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *Int. J. Antimicrob. Agents* 37:576–579. <https://doi.org/10.1016/j.ijantimicag.2011.01.015>.
- Veh, K. A., R. C. Klein, C. Ster, G. Keefe, P. Lacasse, D. Scholl, J.-P. Roy, D. Haine, S. Dufour, B. G. Talbot, A. O. B. Ribon, and F. Malouin. 2015. Genotypic and phenotypic characterization of *Staphylococcus aureus* causing persistent and nonpersistent subclinical bovine intramammary infections during lactation or the dry period. *J. Dairy Sci.* 98:155–168. <https://doi.org/10.3168/jds.2014-8044>.
- Vrieling, M., E. M. Boerhout, G. F. van Wigcheren, K. J. Koymans, T. G. Mols-Vorstermans, C. J. C. de Haas, P. C. Aerts, I. J. J. M. Daemen, K. P. M. van Kessel, A. P. Koets, V. P. M. G. Rutten, P. J. M. Nuijten, J. A. G. van Strijp, and L. Benedictus. 2016. LukMF' is the major secreted leukocidin of bovine *Staphylococcus aureus* and is produced *in vivo* during bovine mastitis. *Sci. Rep.* 6:37759. <https://doi.org/10.1038/srep37759>.

- Vrieling, M., K. J. Koymans, D. A. Heesterbeek, P. C. Aerts, V. P. Rutten, C. J. de Haas, K. P. van Kessel, A. P. Koets, R. Nijland, and J. A. van Strijp. 2015. Bovine *Staphylococcus aureus* secretes the leukocidin LukMF' to kill migrating neutrophils through CCR1. MBio 6:e00335. <https://doi.org/10.1128/mBio.00335-15>.
- Wang, S.-C., C.-M. Wu, S.-C. Xia, Y.-H. Qi, L.-N. Xia, and J.-Z. Shen. 2009. Distribution of superantigenic toxin genes in *Staphylococcus aureus* isolates from milk samples of bovine subclinical mastitis cases in two major dairy production regions of China. Vet. Microbiol. 137:276–281. <https://doi.org/10.1016/j.vetmic.2009.01.007>.
- Wilson, G. J., S. W. Tuffs, B. A. Wee, K. S. Seo, N. Park, T. Connelley, C. M. Guinane, W. I. Morrison, and J. R. Fitzgerald. 2018. Bovine *Staphylococcus aureus* superantigens stimulate the entire T cell repertoire of cattle. Infect. Immun. 86:IAI.00505-18. <https://doi.org/10.1128/IAI.00505-18>.
- Yagci, S., G. Hascelik, D. Dogru, U. Ozcelik, and B. Sener. 2013. Prevalence and genetic diversity of *Staphylococcus aureus* small-colony variants in cystic fibrosis patients. Clin. Microbiol. Infect. 19:77–84. <https://doi.org/10.1111/j.1469-0691.2011.03742.x>.
- Yang, D., A. R. Wijenayaka, L. B. Solomon, S. M. Pederson, D. M. Findlay, S. P. Kidd, and G. J. Atkins. 2018. Novel insights into *Staphylococcus aureus* deep bone infections: The involvement of osteocytes. MBio 9:1–10. <https://doi.org/10.1128/mBio.00415-18>.
- Zadoks, R. N., J. R. Middleton, S. McDougall, J. Katholm, and Y. H. Schukken. 2011. Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. J. Mammary Gland Biol. Neoplasia 16:357–372. <https://doi.org/10.1007/s10911-011-9236-y>.
- Zheng, S. Y., B. Yu, K. Zhang, M. Chen, Y. H. Hua, S. Yuan, R. M. Watt, B. J. Zheng, K. Y. Yuen, and J. D. Huang. 2012. Comparative immunological evaluation of recombinant *Salmonella Typhimurium* strains expressing model antigens as live oral vaccines. BMC Immunol. 13:54. <https://doi.org/10.1186/1471-2172-13-54>.