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DISCONTOOLS SUPPLEMENT

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# Knowledge gaps and research priorities in *Staphylococcus aureus* mastitis control

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**Summary**

This study assessed knowledge gaps and suggested research priorities in the field of *Staphylococcus aureus* mastitis. *Staphylococcus aureus* infecting the mammary gland remains a major problem to the dairy industry worldwide because of its pathogenicity, contagiousness, persistence in the cow environment, colonization of skin or mucosal epithelia, and the poor curing efficacy of treatments. *Staphylococcus aureus* also constitutes a threat to public health due to food safety and antibiotic usage issues and the potential for bidirectional transmission of strains between humans and dairy animals (cows and small ruminants). Gaps have been identified in (i) understanding the molecular basis for pathogenesis of *S. aureus* mastitis, (ii) identifying staphylococcal antigens inducing protection and (iii) determining the cell-mediated immune responses to infection and vaccination. The recommended priorities for research are (i) improved diagnostic methods for early detection of infection and intervention through treatment or management, (ii) development of experimental models to investigate the strategies used by *S. aureus* to survive within the mammary gland and resist treatment with anti-microbials, (iii) investigation of the basis for cow-to-cow variation in response to *S. aureus* mastitis, (iv) identification of the immune responses (adaptive and innate) induced by infection or vaccination and (v) antibacterial discovery programmes to develop new, more effective, narrow spectrum antibacterial agents for the treatment of *S. aureus* mastitis. With the availability and ongoing improvement of molecular research tools, these objectives may not be out of reach in the future.

**KEYWORDS**

diagnostics, epidemiology, gap analysis, mastitis, *Staphylococcus aureus*, therapy, vaccine

## 1 | INTRODUCTION

Despite considerable research on mastitis of dairy ruminants, the disease still remains a major problem to the dairy industry. The need to control mastitis is driven by multiple considerations including milk quality, producer economic viability, reductions in anti-microbial use and animal welfare. Consumers are demanding dairy products that are wholesome, nutritious and safe and that originate from healthy animals. Mastitis is an inflammation of the mammary gland (MG), and the vast majority of mastitis cases are due to an intramammary infection caused by a microorganism (Bramley & Dodd, 1984). Among the numerous bacteria that cause mastitis, only a few species are prevalent and constitute a real issue. *Staphylococcus aureus* is one of these bacteria that cause problems because of its pathogenicity, contagiousness, persistence in the cow environment, colonization of skin or mucosal epithelia, and

  

poor cure rates associated with current therapies. Consequently, *S. aureus* mastitis is difficult to eradicate from herds. The "five-point control plan" (Neave, Dodd, Kingwill, & Westgarth, 1969) made it possible in principle to rid a herd from *S. aureus* intramammary infections (IMI) and maintain a *S. aureus*-free status for long periods, but implementation and maintenance of the programme can be costly and difficult in practice (Hillerton, Bramley, Staker, & Mckinnon, 1995; Zadoks, Allore, Hagenaars, Barkema, & Schukken, 2002). Furthermore, outbreaks of *S. aureus* mastitis can occur in herds that have successfully implemented the five-point plan (Smith, Fox, & Middleton, 1998). In some herds, the classical control programme also appears to be ineffective because of the occurrence of infections by *S. aureus* strains of type patterns similar to that of environmental pathogens (Sommerhäuser et al., 2003). *Staphylococcus aureus* mastitis is defined as an inflammation of the mammary gland caused by infection with usually one, but sometimes several, *S. aureus* strains. There is a wide range of *S. aureus* strains that can cause mastitis (Zadoks, Middleton, McDougall, Katholm, & Schukken, 2011). While most herds have a predominant (contagious) strain-type, less prevalent strains can exist in the same herd (Middleton, Fox, Gay, Tyler, & Besser, 2002a). *Staphylococcus aureus* isolates from mastitis cases have traits that suggest they are adapted to dairy ruminants and possibly to the mammary niche (Peton & Le Loir, 2014). Nevertheless, they can share some pathogenicity attributes with strains of human origin and, more importantly, have the potential to exchange antibiotic resistance determinants with them. Considering their economic impact, and food security and antibiotic usage issues, there is a need to improve the available tools used to control *S. aureus* mastitis. There have been a great many studies of *S. aureus* mastitis of dairy ruminants, both experimental and observational, and a great deal of knowledge has accumulated. Despite all of this research, many knowledge gaps persist, and there is still a need for an improved understanding of key components that determine the limited efficacy of current control methods. Mastitis results when host innate and adaptive defences fail to thwart the invasion and establishment of infection by staphylococci that come in contact with the teat end. Teat end contamination can occur during milking (contagious transmission) or between milkings from the environment. As such, mastitis can be viewed as a disease of management that manifests in the cow. Hence, implementation of mastitis control programmes such as the five-point mastitis control programme (Neave et al., 1969) and the NMC ten-point mastitis control programme have been important in reducing the prevalence of *S. aureus* mastitis. It follows that improved implementation of control procedures through understanding farmer motivations and improving communication is important to reducing the incidence of mastitis (Ritter et al., 2017). Nevertheless, the focus of this review will be on the current understanding of *S. aureus* mastitis disease pathogenesis, epidemiology, diagnosis, treatment and prevention with a view to identifying required innovations and tools to diagnose and control the disease.

## 2 | DESCRIPTION OF THE DISEASE IN THE NATURAL HOST

### 2.1 | Species involved and disease manifestations

*Staphylococcus aureus* causes a variety of diseases in man and animals (Peton & Le Loir, 2014). Mastitis is the main disease caused by *S. aureus* in ruminants, including cows, sheep, goats, camels and water buffalo (Anderson, 1983). *Staphylococcus aureus* is also the predominant pathogen associated with breast abscesses in people (Branch-Elliman et al., 2013). In addition to ruminants, many other animal species can be affected by *S. aureus*, including horses, pigs, dogs, cats, rabbits and poultry (Fitzgerald & Holden, 2016). While infection occurs in many mammalian hosts, asymptomatic carriage is also observed in most species and is usually more prevalent than infection.

In dairy cows, *S. aureus* mastitis is commonly subclinical, manifested by elevated concentrations of leucocytes (primarily neutrophils) in milk (elevated somatic cell counts, SCC). Most infections are chronic, frequently persisting over the ongoing lactation and possibly the following lactations, with more or less intense clinical flare-up episodes. The infection may begin with an acute clinical phase in which there is an elevation of body temperature and a degree of anorexia. This is concomitant with a sharp influx of leucocytes in MG secretions and usually precedes the appearance of clots in milk (Anderson, 1982). Thereafter, the clinical signs (swelling, firmness, warmth, tenderness) are confined to the udder and in a few days they may abate with the apparent milk changes disappearing so that the condition becomes subclinical. Alternatively, infections may colonize udders without clinical signs and spread furtively in the herd. Clinical mastitis in goats and sheep is relatively infrequent, but is generally more severe than in cows (Bergonier, de Cremoux, Rupp, Lagriffoul, & Berthelot, 2003; Contreras et al., 2007). Peracute (severe clinical) mastitis is characterized by hyperthermia, anorexia, rapid heart rate and profound depression, signs that are usually sudden in onset. In the most severe cases, patches of blue discoloration caused by ischaemic gangrene appear, preferentially at the base of the udder and around the teat. If death from toxaemia does not occur or ethical euthanasia is not performed, the affected tissue sloughs from the udder.

It is established that the severity of disease is determined by both the strain virulence and the host condition, but it is unknown to what extent manifestations (subclinical, mild, moderate, severe) and duration of infection are driven by host or pathogen (Fournier et al., 2008; Guinane et al., 2008; Haveri, Taponen, Vuopio-Varkila, Salmenlinna, & Pyorala, 2005; Le Marechal et al., 2011; Plommet & Le Gall, 1963; Postle, Roguinsky, & Poutrel, 1978; Taponen, Liski, Heikkilä, & Pyörälä, 2017). The spread of infections within herds is also under the influence of strain contagiousness and hygiene practices as established by epidemiological studies.

## 2.2 | Incubation period and shedding kinetic patterns

Experimentally induced infections even with low inoculum (<1,000 colony-forming units [cfu]) exhibit short incubation periods (12–48 hr) with most strains (Bannerman et al., 2004; Riollet, Rainard, & Poutrel, 2000). Incubation time is likely dependent both on the infected host and the infecting strain. Field monitoring suggests that the incubation period until clinical signs is variable (days to weeks). Most infections are chronic with varying degrees of bacterial shedding (concentration of viable bacteria) in milk. Shedding is almost continuous but with irregular, cyclical patterns and low numbers in many subclinical cases (Sears, Smith, English, Herer, & Gonzalez, 1990). Consequently, the sensitivity of a single milk sample to allow determination of the infection status of a gland is not perfect, particularly when employing a typical sample volume of 10 μl. A second or third sample for bacterial culture is necessary to reach a high sensitivity of >95%, owing to the irregular pattern of *S. aureus* shedding in milk (Dodd & Neave, 1970; Zecconi, 2010), but this is not practical for herd monitoring programmes. Alternatively, freezing and thawing, incubation before plating, or centrifugation of the milk samples and culturing of the sediment have been shown to improve the detection of *S. aureus* (Artursson, Nilsson-Ost, & Persson Waller, 2010; Godden, Jansen, Leslie, Smart, & Kelton, 2002; Sol, Sampimon, Hartman, & Barkema, 2002; Zecconi, Piccinini, Zepponi, & Ruffo, 1997). Spontaneous cure with cessation of shedding does occur, although in a small percentage (<20%) of infection cases as confirmed by several consecutive samplings (Dodd & Neave, 1970).

## 2.3 | Mechanisms of pathogenicity

The pathogenesis of *S. aureus* IMI has been thoroughly reviewed (Sutra & Poutrel, 1994), and only a few salient considerations will be recalled here. Intramammary infection begins when *S. aureus* passes through the teat canal. Experiments involving experimentally induced infections have shown that very few cfu are necessary to induce infection, (Newbould & Neave, 1965). Moreover, the rate of success and degree of severity are independent of the inoculum size when in the range of 20–200 cfu, provided the strain was a genuine mastitis isolate, the inoculum correctly prepared and the gland free of infection and inflammation (Poutrel & Lerondelle, 1978). Of prime importance is the cell count at time of inoculation (Postle et al., 1978; Schukken et al., 1999). This indicates that the healthy MG is very susceptible to *S. aureus* infection, and suggests that mastitis isolates are very well adapted to their infection niche. Penetration into the MG is thought to occur primarily at or just after milking. At the early stage of infection, the capacity of strains to adhere to the intact epithelium may contribute to establishment of infection (Baselga, Albizu, & Amorena, 1994). Bacteria proliferate in milk and disseminate in a haphazard manner in the cisterns and throughout the duct system. When they reach a threshold concentration, they are detected by the intramammary epithelium which triggers an inflammatory reaction characterized by the influx of neutrophils into the gland tissue and lumen (Le Gall & Plommet, 1965). Direct interaction of bacteria with the epithelium but also released and secreted bacterial products acting as microbe-associated molecular patterns (MAMPs) contributes to the detection of bacteria by the immune system in the MG (Gilbert et al., 2013; Yang et al., 2008).

When neutrophil recruitment is slow or impaired, severe and even gangrenous mastitis can develop (Schalm, Lasmanis, & Jain, 1976). Initially, the *S. aureus* infection is a duct disease, but rapidly the secretory alveoli are involved in lactating glands (Anderson, 1982). Growing staphylococci cause damage to the epithelium of cistern and ducts and then of alveoli. A variety of responses can be elicited, usually but not always involving an initial clinical stage, before evolution towards a chronic subclinical infection with sporadic clinical episodes. Adhesion and invasion of epithelial cells are thought to be instrumental in the establishment of chronic infections (Kerro Dego, van Dijk, & Nederbragt, 2002).

Haemolysins and enzymes are implicated in injuring the epithelium lining. Staphylococci can then adhere to the basal membrane and extracellular matrix using their numerous adhesins (Anderson, 1976). A feature of chronic mastitis histopathology is that it is not homogeneous. Only small areas of udder exhibit infection and inflammatory changes, and the chronic changes are at different stages of evolution throughout the gland, as the infection spreads slowly within the gland (Anderson, 1982). The outcome of interaction of bacteria with neutrophils probably determines the evolution of the infection focus. When the focus is not cleared, the surrounding parenchyma evolves towards involution and fibrosis. A focus of necrosis may appear, as an early stage of parenchymal abscess formation. This evolution is more frequent in small ruminants than in cows. In chronic or recurrent mastitis, the inflammatory response may result both from the direct effect of *S. aureus* MAMPs and a cell-mediated immune response of the delayed type hypersensitivity to *S. aureus* antigens (Targowski & Berman, 1975). Resistance to phagocytosis appears to be a crucial element of *S. aureus* pathogenicity, and *S. aureus* is equipped with many evasion systems aimed at interfering with opsonization, phagocytosis and intracellular killing (Foster, 2005; van Kessel, Bestebroer, & van Strijp, 2014). Specifically, secretion of the bovine-specific leukotoxin LukMF' has been shown to protect the organism from phagocytosis, resulting in more severe clinical signs (Vrieling et al., 2015, 2016). This occurs in vivo when bacterial concentrations exceed $10^6$ cfu/ml, enabling substantial leukotoxin and α-toxin production and leading to the gangrenous form of mastitis (Anderson, 1976; Rainard, 2007).

*Staphylococcus aureus* mastitis isolates have the capacity to produce several surface exopolymers, such as capsular polysaccharides and poly-N-acetyl-β-1,6 glucosamine (PNAG), which are involved in resistance to phagocytosis (Kampen, Tollersrud, & Lund, 2005; Perez et al., 2009; Sutra & Poutrel, 1994). A small proportion of staphylococci ingested by phagocytic cells survive and likely contribute to dissemination in the host. *Staphylococcus aureus* is also ingested by non-professional phagocytes by a mechanism independent of opsonins (Sinha & Fraunholz, 2010). This has been documented in vitro

with mammary epithelial cells (Bayles et al., 1998) and in cells shed in milk of infected quarters (Hebert, Sayasith, Senechal, Dubreuil, & Lagace, 2000). The production of PNAG and invasion of epithelial cells in association with small colony variants (SCV) have been the subject of particular attention (Atalla et al., 2010; Baselga et al., 1994). It is supposed that these defensive forms of growth and survival contribute to the chronic, subclinical nature of many *S. aureus* IMI and intermittent shedding of bacteria (Melchior, Vaarkamp, & Fink-Gremmels, 2006).

Although we know a great deal about *S. aureus* mastitis pathogenesis, all features of *S. aureus* which make this pathogen a successful parasite of the mammary gland have not been clearly identified. There is still a great deal to be learned about host–pathogen interaction of *S. aureus* in the context of mastitis. As an example, it is unclear whether the difference in disease severity between cows, goats and sheep primarily results from differences in virulence repertoire of the causative strains, the host immunology or the host–pathogen interaction. It seems that strains isolated from sheep and goats are related and different from the lineages associated to cows, with a tendency to produce toxins in higher amounts (Bar-Gal et al., 2015; Merz, Stephan, & Johler, 2016; Peton & Le Loir, 2014; Rainard, Corrales, Barrio, Cochard, & Poutrel, 2003), but the pathogenicity of strains of small ruminant origin for the cow udder remains to be established. Also, based on experimental infections with a given strain and inoculum size, there seems to be tremendous animal-to-animal variation in the time-course and severity of the disease (Plommet & Le Gall, 1963). In particular, early events taking place during the lag phase separating the intrusion of staphylococci from the onset of inflammatory response are not well understood. Gaining insight into the temporal expression of the various virulence factors as infection progresses would be useful. This has a bearing on adaptation of *S. aureus* to the udder microenvironment and on in vivo expression of fitness and virulence genes. Also, the proportion of intracellular bacteria that survive, their escape from the phagolysosome and the propensity of cytosolic bacteria to adopt the SCV phenotype are unresolved issues (Sinha & Fraunholz, 2010). The abundance of data from in vitro experiments contrasts with the paucity of evidence based on in vivo or ex vivo data (e.g., pathology specimens), as far as adhesion to epithelial cells, epithelial cell invasion, intracellular survival in phagocytes, the occurrence of microcolonies embedded in slime or the contribution of SCV to resistance to anti-microbial treatments are concerned.

## 3 | EPIDEMIOLOGY

### 3.1 | Pathogen characteristics

*Staphylococcus aureus* is a commensal and opportunistic pathogen of humans and several animal species, including cattle and small ruminants. Evidence of adaptation of mammary-associated isolates to host species comes from the acquisition of genes that encode proteins that target cattle-specific (and small ruminant-specific) molecules and loss or decay of genes that encode proteins adapted to human targets (Guinane et al., 2010; Herron-Olson, Fitzgerald, Musser, & Kapur, 2007). A limited number of lineages, such as those defined by multilocus sequence typing (MLST), are associated with the mastitis isolates, as compared to the higher number of human-associated lineages (Fitzgerald, 2012). Some clonal complexes are common to human and dairy ruminants, others are more specific to dairy ruminants, such as CC133 for goats, or CC97 which represents one of the dominant bovine clones worldwide (Smith et al., 2005; Spoor et al., 2013). Because of the exchange of genetic material between strains by horizontal transfer, the diversity of mastitis-associated isolates is very high. For the same reason, the emergence of increasingly virulent and resistant strains or stealthy and contagious strains that could severely affect agriculture can be anticipated (Lindsay, 2010). This is because many virulence- and resistance-associated genes are borne by mobile genetic elements (MGE). The virulence genes are likely to be involved in different stages of mastitis pathogenesis. The presence of certain virulence-associated genes may be high in certain collections of isolates, and almost absent from isolates collected from other regions of the world, for example, superantigen genes (Adkins, Middleton, & Fox, 2016; Fournier et al., 2008). It does not appear that any virulence-associated gene identified up to now is a requisite for *S. aureus* mastitis isolates to induce an IMI. Instead, different combinations of genes are likely to account for the ability of a strain to induce IMI, as shown for strains of human origin (Peacock et al., 2002).

Another layer of diversity results from the adaptation of *S. aureus* to its environment. Phenotypic diversity can result from the regulation of gene expression (Bronner, Monteil, & Prevost, 2004). In particular, the exoproteome including many virulence factors differs widely, not only due to genome variations, but also because of a very high variability in gene expression (Wolf et al., 2011; Ziebandt et al., 2010). Phase variation influences also the expression of certain disease-associated phenotypes such as the so-called SCV phenotype and intracellular persistence (Tuchscherr, Loffler, Buzzola, & Sordelli, 2010), or slime production and adhesion to or invasion of MEC (Baselga et al., 1993; Cucarella et al., 2002). The expression of capsular material is also subject to regulation, and mastitis isolates have been shown to express various amounts of capsular polysaccharide types 5 or 8, resulting in heterogeneous populations (Poutrel, Rainard, & Sarradin, 1997). Another capsular serotype (type 336) has been proposed and found frequently among *S. aureus* mastitis strains (Guidry et al., 1998), but the corresponding antigen was shown to be teichoic acid (Verdier et al., 2007). A high proportion of strains do not produce a detectable amount of capsular polysaccharide and appear as non-typeable, but carry an intact capsule gene cluster (Tollersrud, Kenny, Reitz, & Lee, 2000). Loss of capsular expression has also been found and suspected to be associated with persistence of infection (Tuchscherr et al., 2010).

In keeping with their genetic and phenotypic diversity, mastitis isolates are known to differ in pathogenicity. An association between mastitis severity and strain-type has been found (Haveri et al., 2005; Matsunaga, Kamata, Kakiichi, & Uchida, 1993; Zadoks et al., 2000); however, another study evaluating cases of subclinical mastitis

demonstrated cow-to-cow variation in milk SCC response to different strains with no significant difference in SCC between strains under field conditions in eight herds (Middleton et al., 2002a). The capacity of certain strains to establish persistent intramammary infections with a higher success rate than other strains has been documented under experimental conditions (Postle et al., 1978). In addition, some strains that have been used on several occasions in experimental studies proved to induce either acute or subacute mastitis (Bannerman et al., 2004; Poutrel & Lerondelle, 1978; Riollet et al., 2000). Differences in capacity to spread from cow-to-cow within a herd (contagiousness), or in capacity of certain strains, or strains of a given genotype, to spread in spite of implemented control practices have also been documented (Fournier et al., 2008; Graber et al., 2009; Smith et al., 1998). Taken together, these data suggest that while strain may be important in disease severity, there are also cow factors most likely related to innate and adaptive immune responses that influence disease outcomes.

Many studies have brought in a wealth of knowledge on epidemiological characteristics of strains (contagiousness, clinical expression and flare-up rates, curability). Still more progress in the understanding of the epidemiology of *S. aureus* mastitis can be expected using modern genotyping approaches such as whole-genome sequencing and other molecular tools applied on a large-scale basis to determine the infection dynamics within and between herds.

## 3.2 | Host range and zoonotic potential

Concurrently with domestication, several host jumps from human to bovids occurred in the past and from bovid to human hosts more recently (Weinert et al., 2012). *Staphylococcus aureus* frequently colonize the skin and nasal passages of humans (Kuehnert et al., 2006) and in susceptible people can cause a variety of pathologies ranging from skin and soft tissue infections to endocarditis and osteomyelitis. While human and bovine *S. aureus* strains are usually regarded as distinct from each other (Larsen et al., 2000; Schlegelova, Dendis, Benedik, Babak, & Rysanek, 2003), work dating back to the 1960s suggests that humans and cattle can share the same strains via direct contact (Davidson, 1961). Historical data suggested that *S. aureus* shared between humans and animals most likely came from humans, that is, correspond to an anthroponosis (Davidson, 1961; Devriese & Hommez, 1975). More recently, evidence supporting zoonotic transmission, particularly with MRSA strains, has been reported (Garcia-Alvarez et al., 2011; Holmes & Zadoks, 2011). The emergence of clones of bovid origin that switched to humans, adapted to their new host and spread in global human populations has been reported (Spoor et al., 2013). A few strains isolated from bovine mastitis or from bovine milk are shared with humans, such as some presumed bovine-adapted methicillin-resistant strains (Garcia-Alvarez et al., 2011). The recent emergence of livestock-associated methicillin-resistant (LA-MRSA) S. *aureus* of the ST398 shared by pigs, cattle and humans has led to suggestions that the strain may be spreading in dairy herds (Harrison et al., 2013; Vanderhaeghen et al., 2010). It is thus possible that the zoonotic risks linked to *S. aureus* mastitis will become an issue in the future (Zadoks et al., 2011).

Exchange of MGE between human and bovine strains is also a concern. The fact that coagulase-negative staphylococci, the most common bacteria isolated from ruminant milk, can frequently carry anti-microbial resistance genes such as *mecA* that could transmit to *S. aureus* is also a concern (Holmes & Zadoks, 2011), and surveillance of mastitis pathogens for anti-microbial resistance genes is required. Transmission to milking personnel by direct contact with infected dairy ruminants probably occurs but its frequency is unknown. Presently, as most strains of ruminant origin are not well equipped to induce disease in humans, the occurrence of disease in humans as a result of direct transmission from milk to humans is likely to be rare. Nevertheless, there is a need for continued epidemiological surveillance for emergence of strains common to both ruminants and humans, and a more in-depth understanding of the flow of strains between humans and ruminants as well as the potential for zoonotic transmission of *S. aureus* from ruminants via unpasteurized milk. The development of new technologies enabling rapid, inexpensive and high-throughput sequencing for whole genome, supported by standardized methodology and a recording and reporting infrastructure, makes this surveillance possible (McAdam, Richardson, & Fitzgerald, 2014).

Staphylococcal enterotoxins pose another threat for public health. A notable proportion of food poisoning cases is due to enterotoxigenic *S. aureus* contaminating milk or milk products (Le Loir, Baron, & Gautier, 2003). Part of these contaminations results from shedding of *S. aureus* by infected mammary glands. Several studies have found that the majority of *S. aureus* mastitis isolates carry at least some of the enterotoxin-encoding genes, although with a wide variation in prevalence and enterotoxin profile (Larsen, Aarestrup, & Jensen, 2002; Mello et al., 2016; Ote, Taminiau, Duprez, Dizier, & Mainil, 2011). Foodborne infection risk is low in the countries where pasteurization is applied to most milk products, but there exists a risk with raw milk and products made with raw milk. Enterotoxigenic strains need to grow to concentrations $>10^5$ cfu/g before the toxin is produced at detectable levels. Accordingly, cheese batches made from raw milk are tested for presence of coagulase-positive staphylococci and tested for staphylococcal enterotoxins whenever cfu levels exceed $10^5$ cfu/g (EU regulation EC 2073/2005). Of note, enterotoxins are resistant to heat, freezing and irradiation. Hence, toxins produced before heat-treatment are extremely difficult to eliminate from foods and can cause intoxication.

## 3.3 | Reservoirs, transmission and vectors

Chronically infected mammary glands represent the main reservoir of *S. aureus* in herds. Nevertheless, *S. aureus* can colonize other body sites like the teat and inguinal skin, nares and hocks particularly when wounded (Capurro, Aspan, Ericsson Unnerstad, Persson Waller, & Artursson, 2010). The nasal cavity may represent the primary reservoir of *S. aureus* in sheep flocks (Mork, Kvitle, & Jorgensen, 2012). Moreover, *S. aureus* can survive for some time in the dairy

cow environment including bedding materials, and on milking equipment and facilities (Roberson, Fox, Hancock, Gay, & Besser, 1994). Based on epidemiological studies, results of mastitis control programmes and molecular data, *S. aureus* is classified as a contagious pathogen. Nevertheless, the frequent occurrence of multiple strains with low prevalence or incidence in infected herds indicates that not all infections are the result of cow-to-cow transmission (Zadoks et al., 2011). In most infected herds, one or two prevalent strains affect multiple cows (Middleton et al., 2002a; Zadoks et al., 2000). The pathogen is primarily transmitted during the milking process as the bacteria are spread to uninfected quarters by teat cup liners, milkers' hands, and wash cloths (fomites). Yet heifers are frequently infected at first calving, although they are not exposed to the milking machine or the milking process, which is thought to be the main source of *S. aureus*. Flies have been shown to be colonized and act as possible vectors for the transmission of *S. aureus* in cases of bovine mastitis (Anderson et al., 2012; Owens, Oliver, Gillespie, Ray, & Nickerson, 1998). In sheep and goats, the contaminated mouth of suckling lambs or kids may present another transmission route, but this has not been substantiated. The roles of extra-mammary colonization of healthy persistent carriers and of environmental sources as a reservoir for intramammary infection are not well defined. While some evidence demonstrates body site colonization as a risk factor (Roberson et al., 1994) and that *S. aureus* strains that cause mastitis can originate from other cows or the environment (Sommerhäuser et al., 2003), a complete understanding of the relationship between colonization and what drives the shift from colonization to IMI still needs to be investigated using modern molecular epidemiological methods. We also need to understand fully the mechanisms of transmission of *S. aureus* between humans and dairy animals (cows and small ruminants). Improved biosecurity and hygiene control measures may limit opportunities for livestock-to-human transmission.

### 3.4 | Geographic distribution and spread

As a consequence of its contagiousness and capacity to induce long-lasting chronic infections, *S. aureus* is among the few major pathogens associated with endemic mastitis all over the world (Abera, Habte, Aragaw, Asmare, & Sheferaw, 2012; Acosta, da Silva, Medeiros, Pinheiro, & Mota, 2016; Levison et al., 2016; Petzer, Karzis, Watermeyer, van der Schans, & van Reenen, 2009; Piehler, Grimholt, Ovstebo, & Berg, 2010; Taponen et al., 2017; Wang et al., 2015). The prevalence of *S. aureus* mastitis has been reduced in countries or regions that implement the standard mastitis prevention programme (Neave et al., 1969). Nevertheless, because of imperfect or discontinued implementation and of resistance of the bacteria to treatment, the prevalence of *S. aureus* mastitis in cows and small ruminants remains consequent in many countries (Botrel et al., 2010; Contreras et al., 2007; Dore et al., 2016; Tenhagen, Koster, Wallmann, & Heuwieser, 2006; USDA-APHIS, 2008).

The herd is the epidemiological unit. Dairy cattle herds can be free of *S. aureus* intramammary infections for long periods. Ovine and caprine flocks are seldom completely free of *S. aureus* infection, and clinical mastitis cases appear from time-to-time. In principle, spread between herds should not be a major problem. Spread occurs mainly by introduction of an infected animal and could be prevented with a few appropriate biosafety measures. Speed of spread within herds can be high, depending mainly on hygienic precautions implemented in the herd and the virulence and transmissibility of the prevalent strains (Voelk et al., 2014). New practices could increase the risk of spread. For example, in some regions, there has been a move from closed herds to the use of contracted heifer farms that supply heifers to dairy farms. One study showed that herds that purchased replacement heifers had a higher prevalence of *S. aureus* than herds that purchased replacement lactating cows for expansion, and had more total strains of *S. aureus* and more new strains than closed herds that reared their own replacements (Middleton, Fox, Gay, Tyler, & Besser, 2002b). The movement of cattle between farms is much higher than 20–30 years ago and needs to be further evaluated as a practice.

Epidemiological surveys of bacteria responsible for clinical and subclinical mastitis are necessary worldwide to monitor the changing prevalence of *S. aureus* and the importance of this pathogen as agent of mastitis. Global analysis of the evolution and geographic spread of strains and the identification of newly emerging strains using powerful genomic approaches is now possible and desirable.

## 4 | SOCIO-ECONOMIC IMPACT

The true socio-economic impact of *S. aureus* mastitis has not been fully ascertained but *S. aureus* as a mastitis pathogen of ruminants impacts animal health, well-being and productivity of quality milk and, consequently, farm income. Moreover, *S. aureus* may also impact human health due to potential zoonotic transmission. By impeding the economic viability of the producer, mastitis has the potential to limit investments in improving herd performance. Not only does mastitis have an impact on cow productivity and milk quality, but it may shorten the cow's lifespan in the herd, which may also impact the herd's ability to genetically improve. In general, costs associated with mastitis include milk production losses, pharmaceuticals, discarded milk, veterinary services, labour, milk quality deficits, investment in mastitis management protocols and infrastructure, diagnostic testing and cattle replacement (Halasa, Huijps, Osteras, & Hogeveen, 2007). According to a comprehensive review on the overall economic effects of bovine mastitis and mastitis management (Halasa et al., 2007), the cost per case of clinical mastitis was estimated at 287 and 102 per case of subclinical mastitis. In a UK study, the estimated annual output losses, treatment costs and costs of prevention for mastitis were £197.9 million, £79.8 million and £9.3 million, respectively (Bennett, Christiansen, & Clifton-Hadley, 1999). Swinkels and co-authors developed an economic model to determine the benefits of lactational therapy of subclinical *S. aureus* mastitis (Swinkels, Hogeveen, & Zadoks, 2005). This analysis determined that when contagious transmission of *S. aureus* was high in a herd, the economic benefit of lactational therapy was 95.6 and 142.4 for

3 day and 8 day treatment regimens, respectively. Conversely, the economic benefit in low transmission herds was -21.1 and -57.7 for the same treatment regimens. However, these authors concluded that the economic outcome of lactational therapy for subclinical *S. aureus* mastitis is dependent on herd, cow and strain differences. Duration of infection is an important consideration with regard to treatment efficacy and animals with indurated tissue, multiple quarters infected or other indicators of persistent infection should not be selected as treatment candidates. To illustrate this point, Barkema and co-authors (Barkema, Schukken, & Zadoks, 2006) concluded that "treatment of young animals with penicillin-sensitive *S. aureus* infections is often justified based on bacteriological cure and economic outcome, whereas treatment of older animals, chronic infections, or penicillin-resistant isolates should be discouraged."

Direct mortality due to *S. aureus* mastitis in dairy herds is usually low, but indirect losses resulting in premature culling due to chronic incurable cases of *S. aureus* mastitis can be high in problem herds. Direct mortality from peracute gangrenous mastitis, while more common in sheep and goats (Contreras et al., 2007), can occur in cattle. Hence, while less common, clinical *S. aureus* mastitis can have obvious animal welfare implications.

## 5 | *STAPHYLOCOCCUS AUREUS* MASTITIS IMMUNOBIOLOGY

There is an abundance of literature on the interaction of mastitis-associated *S. aureus* with the immune system of ruminants, but in this article, the focus will be on some aspects which point to research priorities. Regarding the classical subclinical and chronic type of *S. aureus* IMI, a striking observation is that there is no evidence of protection by a previous case of infection in cows under field conditions (Cha et al., 2016; Zadoks et al., 2001). The capacity for experimentally infecting the same quarter of a cow subsequently with a different strain of *S. aureus* or with the same strain strongly suggests that infection does not induce the level of protection necessary to allow the MG to eliminate the bacteria (Postle et al., 1978; Sutra & Poutrel, 1994). Lack of naturally acquired full protection renders more difficult the identification of protective immune mechanisms. Yet infection elicits an immune response, as rising antibody titres against bacterial antigens can be detected (Loeffler & Norcross, 1985). In particular, antitoxins are induced, and they are likely to be instrumental in the reduced severity of subsequent infections by toxin-producing strains. Specifically, antibodies to haemolysins and leucotoxins are very likely to reduce the severity of clinical mastitis (Plommet & Vidal, 1963; Rainard, 2007). There is a paucity of data on naturally acquired antibodies to exopolymers (capsule and PNAG). Such antibodies are supposed to help phagocytes to ingest and kill staphylococci. There are also in vitro studies showing that they reduce the adhesion to epithelial cells and subsequent invasion (Cifrian, Guidry, O'Brien, & Marquardi, 1996; Renna et al., 2014). Of note, most adult cows have high titres of opsonic antibodies to mastitis-associated *S. aureus* strains in their blood, mainly in the IgM isotype (Williams & Hill, 1982). Deposition of complement on mastitis isolates is not a requisite for efficient opsonization (Barrio, Rainard, & Poutrel, 2003).

It is undisputed that a major protective response is phagocytosis and killing of staphylococci by neutrophils. The prompt recruitment of neutrophils to the mammary infection sites is of prime importance, along with the help of opsonins (antibodies and complement) and of activating cytokines produced by a variety of myeloid and lymphoid immune cells that support the neutrophil defence system (Burton & Erskine, 2003). That this system is important is supported by the array of the staphylococcal factors designed to counter it, such as exopolymers, staphylococcal protein A, alpha-toxin, leucotoxins and others (Foster, 2005; van Kessel et al., 2014).

There are indications that the inflammatory response induced in cows by *S. aureus* is blunted compared to the full-blown inflammation triggered in the MG by *Escherichia coli*. In particular, milk concentrations of chemoattractants for neutrophils (IL-8, C5a) and of TNF-$\alpha$, a potent activator of neutrophil activity, are much lower, whereas concentrations of the anti-inflammatory cytokine TGF-$\beta$ are comparable (Bannerman, 2009). Consequently, the cytokine milieu may not be optimal for the full expression of neutrophil bactericidal potential, with consequences in terms of intracellular survival and disease persistence (Anwar, Prince, Foster, Whyte, & Sabroe, 2009). Sensing of *S. aureus* by the MG is likely to involve cells of the epithelial lining sensu lato, that is, epithelial cells and macrophages. Mammary epithelial cells react in vitro to killed or live *S. aureus* (Lahouassa, Moussay, Rainard, & Riollet, 2007; Yang et al., 2008) or to MAMPs such as lipoteichoic acid or peptidoglycan fragments, and these responses tend to correlate with the response of the MG to intramammary infusion of MAMPs (Bougarn et al., 2010). The milder response of epithelial cells to *S. aureus* when compared to *E. coli* or to endotoxin is in line with the usually milder severity of infections by these pathogens (Gilbert et al., 2013; Günther et al., 2011; Strandberg et al., 2005).

The role of cell-mediated immunity in MG defence has been overshadowed by the traditional focus on toxin-neutralizing and opsonizing antibodies, as exemplified by the absence of cell-mediated adaptive immune response section in a recent comprehensive review (Schukken et al., 2011). There is currently an increasing interest in the T-cell response to *S. aureus* infections (Broker, Mrochen, & Peton, 2016). Different subpopulations of T cells are likely to contribute to anti-staphylococcal immune defence. Th17 cells are specialized in the triggering of neutrophilic inflammation at epithelial sites and defence against extracellular bacteria, mainly through production of the cytokine IL-17A (Iwakura, 2008). Expression of the gene encoding IL-17A has been found in milk somatic cells of quarters infected by *S. aureus* (Tao & Mallard, 2007), and numbers of Th17 cells increase in the MG in the course of a mouse model of *S. aureus* mastitis (Zhao et al., 2015). As immunization can induce a Th17-mediated recruitment of neutrophils in the bovine MG and mammary epithelial cells respond synergistically to IL-17A and staphylococcal MAMPs (Bougarn et al., 2011; Rainard et al., 2015), it can be speculated that inducing a protective Th17-type immune response by vaccination is possible.

Other T cells that could contribute to MG defence are CD8 T cells. There are many CD8 T cells in subepithelial position in the MG, and they are recruited in milk of healthy glands or in response to *S. aureus* MG infection, but their roles remain obscure (Park, Fox, Hamilton, & Davis, 1992; Riollet, Rainard, & Poutrel, 2001; Soltys & Quinn, 1999). Considering the possible role of *S. aureus* intracellular survival in infection persistence, induction of cytotoxic CD8 T cells could well be a key vaccine-induced immune response.

Another aspect of cell-mediated immunity, which has hardly been taken into account until now, is the occurrence of regulatory T cells as a component of the immune response to chronic *S. aureus* infection or to vaccination (Park, Fox, Hamilton, & Davis, 1993). During its long history of interactions with its hosts, *S. aureus* has developed tools to interfere with the T-cell immune response, such as the well-known superantigens, but also less well understood misguiding mechanisms (Broker, Holtfreter, & Bekeredjian-Ding, 2014).

Transcriptomic and proteomic studies have been performed that describe expression of genes and proteins during mammary infection course in cattle and sheep. The immune response against *S. aureus* is different from that against *E.coli* (Ibeagha-Awemu, Ibeagha, Messier, & Zhao, 2010; Lee, Bannerman, Paape, Huang, & Zhao, 2006). In vitro studies using epithelial cell lines or primary cells reproduce some of these differences (Gilbert et al., 2013; Yang et al., 2008). Comparison of gene expression profiles has revealed stronger T cell activation by *S. aureus* compared to coagulase-negative staphylococci (Bonnefont et al., 2011). Other specific transcriptional features remain to be determined.

Overall, local mammary immune responses to infection remain relatively under-studied. Informative techniques should be applied to understand how local and systemic immunity combine to provide protection or favours chronicity following vaccination or during infection.

## 6 | MAIN MEANS OF PREVENTION, DETECTION AND CONTROL

### 6.1 | Biosecurity and sanitary measures

As *S. aureus* mastitis is essentially a contagious disease spreading from infected udders to healthy cows, sanitary measures are essential. Implementation of the standard mastitis prevention programme is usually effective at controlling the disease (Dodd & Neave, 1970; Zadoks, Allore, et al., 2002). The programme involves the proper cleaning and drying of teats before milking, proper use of correctly tuned milking machines, post-milking teat disinfection, use of dry cow therapy, culling of chronically infected cows, milking of infected cows in a separate group and establishing an active milk quality programme. However, in some situations, these measures are inadequate at preventing spread (Smith et al., 1998). Because of a poor efficacy in the treatment of long-lasting chronic infections, the affected cows should be culled from the herd. Appropriate biosecurity measures are of prime importance to avoid re-introducing bacteria in a *S. aureus*-free herd or introducing new lineages in an infected herd. Maintaining a closed herd is desirable. When purchasing cows, if necessary for herd expansion or animal replacement, a number of precautions have to be taken (Barkema, Green, Bradley, & Zadoks, 2009).

### 6.2 | Diagnostics

Routine diagnosis of mastitis is based on determining the concentration of somatic cells in milk, also known as SCC. While *S. aureus* IMI is often associated with chronic elevations in SCC in cows and small ruminants (Koop, Nielen, & van Werven, 2012; Paape et al., 2007; Schukken, Wilson, Welcome, Garrison-Tikofsky, & Gonzalez, 2003), this test is not specific for *S. aureus* IMI as many other bacteria can stimulate the same response. Hence, aetiological diagnosis is only possible based on detection of bacteria in aseptically collected milk samples from the mammary gland. Bacteria can be cultured on growth media or detected using molecular methods such as polymerase chain reaction (PCR).

*Staphylococcus aureus* is easily grown on blood agar at 37°C after 24 hr of incubation (Middleton, Fox, Pighetti, & Petersson-Wolfe, 2017). Blood agar is the preferred medium for routine bacterial culture of milk because it supports growth of a large array of mastitis pathogens and allows detection of complete and incomplete haemolysis (Middleton et al., 2017). The use of selective media, while limiting growth of potential contaminants, does not significantly improve diagnostic accuracy (Zecconi, 2010). The risk of contamination during sample collection in the milking parlour leading to false positives or alternatively the possibility of false-negative test results due to intermittent or low numbers of bacteria in the sample are impediments to the bacteriological diagnosis (Middleton et al., 2017). Further, misdiagnosis due to the SCV being confused with other bacterial genera is possible. Pre-enrichment in liquid broth before isolation, freeze–thawing, increasing the volume of plated milk from 0.01 to 0.1 ml or duplicate quarter milk samples reduces the proportion of false-negative results (Artursson et al., 2010; Buelow, Thomas, Goodger, Nordlund, & Collins, 1996; Godden et al., 2002; Sol et al., 2002; Zecconi et al., 1997). However, cost and, in some instances, turnaround time remains a limitation for implementation of bacteriological techniques on a large scale. To shorten turnaround times, conventional bacteriological methods have been adapted to on-farm use for the detection of multiple mastitis pathogens including *S. aureus* with outcomes being similar to laboratory-based methods (Ganda, Bisinotto, Decter, & Bicalho, 2016).

Polymerase chain reaction can be used to identify bacterial DNA in aseptically collected milk samples (Gillespie & Oliver, 2005). Tests based on bacterial nucleic acid detection and quantification already show promise and deserve further research and development as a method to rapidly, accurately, and cost-effectively, diagnose IMI (Koskinen et al., 2009, 2010; Voelk et al., 2014; Zanardi et al., 2014). Commercially available real-time PCR-based reagent kits for detection of an array of mastitis-causing pathogens, including *S. aureus* and *Staphylococcus* spp., are currently available in the marketplace, but require a significant initial investment in equipment, and

the cost per sample still exceeds routine bacteriological culture. While cycle threshold can give a relative idea of the amount of bacterial DNA in the sample, with higher cycle thresholds indicating lower amounts of bacterial DNA in the sample, current techniques tend to provide a yes/no answer and do not provide proof of life of the bacteria. Hence, new approaches to molecular diagnostics that lower cost and provide evidence of replicating bacteria are needed.

Some more recent developments include the use of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and the loop-mediated isothermal amplification (LAMP) assay to identify *S. aureus*. In its current format, MALDI-TOF requires an isolated bacterial colony and is used to make a genus and species identification. After the initial investment in the machine, cost per sample is quite low. While direct identification of bacteria in milk has been reported (Barreiro et al., 2017), currently *S. aureus* cfu numbers needed in the milk to make an accurate diagnosis with MALDI-TOF far exceed what would be expected to be detected in conventional culture. Hence, further refinement is needed before it is used to directly identify *S. aureus* in milk samples. It was recently reported that the LAMP assay could detect *S. aureus* in milk with results in 2 hr (Sheet, Grabowski, Klein, & Abdulmawjood, 2016); however, the lower limit of detection was reported as 900 cfu/ml, which could lead to false-negative results for cows or mammary quarters shedding low numbers of organisms. In the future, diagnosis of *S. aureus* mammary gland infection might benefit from the detection of miRNA in milk exosomes or blood (Sun et al., 2015).

Several immunoassays have been described, and a few are patented for the identification of *S. aureus* in food or milk (Fabres-Klein, Aguilar, Silva, Silva, & Ribon, 2014). Some of these tests may be suitable for diagnosis of *S. aureus* mastitis, but they still require validation and adaptations for the diagnosis of mastitis on farm. Anti-staphylococcal antibody titres increase as mastitis develops. Pre-existing antibodies against *S. aureus* antigens are present in the serum of uninfected as well as infected cows. In milk from a healthy gland, antibody titres correlate with blood titres, due to transudation of plasma antibodies and preferential transport of IgG1. In infected glands, milk titres depend more on exudation of plasma than on local synthesis. As a result, any inflammation of the mammary gland, caused by any pathogen, may provoke an increase in milk antibody titres to *S. aureus*, a phenomenon which complicates the use of antibodies for immunological diagnosis. Milk antibody concentrations are also impacted by stage of lactation (Fox & Adams, 2000). To find a *S. aureus* antigen inducing antibodies during infection, but not recognized by pre-infection serum (giving rise to sero-conversion), may solve the issue. This goal may be unattainable.

While identification of *S. aureus* infected animals is critical to implementing control strategies, subspecies identification may be necessary to differentiate sporadic strains from highly contagious strains. Ultimately, the most discriminatory means of comparing two or more *S. aureus* isolates to determine strain variation would be to compare whole-genome sequences. While some laboratories have this capability and the budget to support such analyses, it is not currently universally available and cost and time effective. In the absence of capability to conduct whole-genome sequence assembly and comparative analysis, other methods have been used such as pulsed-field gel electrophoresis (PFGE), MLST, multiple locus variable number tandem repeat analysis, spa-typing, RS-PCR, toxinotyping, ribotyping, biotyping and randomly amplified polymorphic DNA (RAPD) PCR (Fournier et al., 2008; Ikawaty et al., 2009; Myllys, Ridell, Bjorkroth, Biese, & Pyorala, 1997; Sobral et al., 2012). Among these techniques, PFGE is regarded as the most discriminatory (Adkins et al., 2016; Ikawaty et al., 2009).

Some of these subspecies identification techniques tend to lack sufficient discriminatory power lumping many strains together suggesting widespread contagiousness, when in fact more discriminatory methods would tell a different story (Adkins et al., 2016; Zadoks, Leeuwen, et al., 2002). Lack of discriminatory power is of concern when culling decisions are being made because animals may be falsely diagnosed with an apparently contagious strain when in fact they could have a sporadic strain. Hence, while these latter techniques may in some cases be more rapid or cost-effective than whole-genome sequence comparison, there is still a need for a rapid, inexpensive method to determine contagiousness of *S. aureus* at the farm-level.

Further development of rapid and sensitive cow-side or in-line pathogen-specific diagnostics as well as cost-effective tools to determine contagiousness, pathogenicity or antibiotic resistance is needed to facilitate treatment and control measures. A screening test for dry cows, non-lactating heifers and latent carriers would help prevent introduction of new highly contagious strains at purchase and improve herd biosecurity. Early detection of IMI through technologies that allow frequent monitoring of IMI status would facilitate treatment within 2 weeks of infection increasing the odds of cure. More knowledge on determinants of highly contagious or multiresistant strains is needed to monitor and identify the occurrence of infections that will be difficult to cure and eradicate, and to evaluate the zoonotic potential of these strains. The biggest hurdle to development of new diagnostics will be minimizing cost per test.

### 6.3 | Therapeutics

Anti-microbial therapy during lactation or the dry period results in real or apparent cure rates that are highly variable (from 4% to 92%), depending on a number of factors including herd transmission rates, cow, pathogen and treatment regimen (Barkema et al., 2006). As to host-level factors, lower probability of cure is associated with ageing of the cow (primiparous vs higher parity), high levels of SCC ($>10^6$ cells/ml), duration of the mammary infection (>2–4 weeks), high bacterial load in milk before treatment, and number (>1) and position (hind quarters) of infected quarters. These factors are helpful for selection of the cows that may benefit from treatment and guide the decision to treat or not. However, herd level factors such as transmission rates may influence the economic justification for lactational therapy of subclinical *S. aureus* mastitis and should also be considered when making treatment decisions (Swinkels et al.,

2005). Pathogen factors also play a role, but with the exception of anti-microbial resistance, they remain poorly defined. Resistance to β-lactam antibiotics is the most well-known antibiotic resistance of *S. aureus* mastitis isolates. It has been shown that the cure rate is lower for penicillin-resistant isolates regardless of the anti-microbial molecule used for treatment (Barkema et al., 2006). The mechanisms underlying the association between β-lactam resistance and poor response to other antibiotic treatment are currently unknown. Testing for anti-microbial susceptibility could then be limited to testing sensitivity to penicillin or β-lactamase production before deciding to treat a group of cows in a herd. Host-adapted strains like those that are grouped together in the clonal complex 97 or other bovine-associated sequence types may be more difficult to cure (van den Borne et al., 2010), possibly owing to their capacity to survive in bovine mammary tissue (Budd et al., 2015). Availability of typing methods, and whole-genome sequencing, may improve our knowledge of specific virulence traits or features that make these strains more difficult to cure.

Treatment success rate is not completely correlated with in vitro susceptibility. Treatment modalities play also a central role. Although *S. aureus* is susceptible to a variety of antibiotics in vitro, biology of staphylococci and adaptation to the bovine host environment make some treatments inefficient. Several factors like the ability of *S. aureus* (i) to reside inside the host cells by surviving the neutrophils arsenal upon phagocytosis or by infecting mammary epithelial cells, (ii) to form small colony variants or L-forms and (iii) to induce formation of (micro-)abscesses and fibrosis are all detrimental to the efficacy of anti-microbial treatment. The intracellular location of *S. aureus* is a contributing factor to the problem of therapeutic failure. A commercially available antibiotic product has been shown to be able to kill *S. aureus* internalized in mammary epithelial cells in vitro, but its superior efficacy to cure chronic mastitis has not been established (Almeida, Patel, Friton, & Oliver, 2007). Another problem is that the intracellular staphylococci are not in a metabolic state of susceptibility to the antibiotic (Craven and Anderson, 1980).

Many treatments have been used for *S. aureus* mastitis, with varying efficacy. Combination of drugs, route of application (mammary versus systemic) and duration of treatment have been used to improve efficacy. There is no real evidence proving that addition of neomycin to penicillin for intramammary treatment improves cure rate (Taponen et al., 2003). Combined treatment by systemic and intramammary routes is not always more effective. If this is further confirmed, the intramammary route should be the rule for treating subclinical and low/moderate clinical *S. aureus* mastitis to limit antibiotic exposure of the digestive flora and prevent spreading of antibiotics resistance. Extended treatment is generally associated with a higher probability of cure (Roy & Keefe, 2012).

Chronic infections that have resisted one or two treatments are considered impossible to cure, and culling is the best solution to reduce the risk of infection spread within the herd (Barkema et al., 2006). As the probability of cure has a large impact on the economic benefit of treatment, cost-benefit analyses are necessary before application of any treatment, including side effects like the persistence of infected cows in a herd as a source of new contaminations. Studies are needed to determine the pathogen factors that affect cure to allow implementation of strategic decisions that cover all the aspects listed above including economic considerations and the further development of alternative treatments or the combination of existing modalities like vaccination and treatment. In the recent past, there was little evidence for an increase in antibiotic resistance among *S. aureus* mastitis isolates, including methicillin resistance. Nevertheless, this may be changing, as multiresistant strains, including MRSA, are appearing in certain countries (Wang et al., 2015). The fact that coagulase-negative staphylococci (the most common bacteria isolated from milk) frequently carry anti-microbial resistance genes, such as *mecA*, that can potentially transmit to *S. aureus* is also of concern. In any case, the prudent use of antibiotics is strongly advocated and the surveillance of mastitis pathogens for anti-microbial resistance genes is a necessity. Commercial potential of new anti-microbial agents is limited by regulatory hurdles and the will to narrow the spectrum of anti-microbials in veterinary medicine to those that are not critical for human use. Most animal health companies have exited antibiotics discovery. The development of new classes of anti-microbial agents that provide high levels of efficacy with minimal human health issues, such as peptide anti-microbials, would be a way to dodge this constraint. Alternative treatments have been proposed, such as bacteriocins, essential oils and other herbal and homoeopathic remedies, but to date, there is lack of scientific evidence that supports recommendations for use.

### 6.4 | Vaccines

Vaccination against *S. aureus* and *S. aureus* mastitis, more specifically, has been studied for many years with very few products making it to market. To be effective, the ideal *S. aureus* mastitis vaccine should either prevent infection or facilitate clearance of the bacteria from the mammary gland very shortly after IMI thus eliminating the possibility of a long-term intramammary infection that can serve as a reservoir for infection of herd-mates. To date, a *S. aureus* mastitis vaccine that meets these criteria has not been developed.

Currently marketed products available for dairy cattle and dairy goats primarily stimulate humoral immunity. While vaccine-induced antibody can be detected in plasma and milk, the levels of opsonizing antibody in milk can be limited (Luby & Middleton, 2005; Middleton, Luby, & Adams, 2009). The majority of data demonstrates that *S. aureus* mastitis vaccines have the most efficacy in decreasing the clinical severity of mastitis with some studies demonstrating a reduction in the rate of new IMI (Middleton et al., 2006; Williams, Mayerhofer, & Brown, 1966; Williams, Shipley, Smith, & Gerber, 1975). When using a commercial *S. aureus* bacterin in replacement heifers at 6 months of age followed by booster vaccinations every 6 months until calving, Nickerson and co-authors (Nickerson, Owens, Tomita, & Widel, 1999) demonstrated a reduction in new IMI during pregnancy and new IMI at calving compared to unvaccinated control heifers, but infections still occurred in some of the vaccinates. Most

recently, a European study using a commercial bacterin showed that, when used in conjunction with a comprehensive contagious mastitis pathogen control programme, vaccination led to a decrease in the duration of IMI and incidence of *S. aureus* IMI in two dairy cattle herds (Schukken et al., 2014), but these positive results were not confirmed in other studies (Freick et al., 2016; Landin, Mork, Larsson, & Waller, 2015).

Experimental and commercial *S. aureus* vaccines have also been studied for their ability to augment intramammary antibiotic therapy (Luby & Middleton, 2005; Sears & Belschner, 1999; Smith, Lyman, & Anderson, 2006; Timms, Kirpatrick, & Sears, 2000). Results varied by study and while enhancement of treatment efficacy was recognized in some herds in some of the studies, results of one of the studies (Luby & Middleton, 2005) using a commercial bacterin around the time of treatment showed no significant increase in cure rate over cows treated with only intramammary antibiotics.

While vaccination against *S. aureus* mastitis has appeal both from the perspective of reducing antibiotic use and preventing chronic IMI that are refractory to treatment, current technologies lack in their ability to stimulate a robust humoral and cell-mediated immune response capable of completely preventing or clearing IMI shortly after infection. If efficacious vaccines are to be pursued, a more thorough understanding of the host–pathogen interaction and immunity to *S. aureus* must be gained. A more practical short-term solution might be to expand on the work of Schukken and co-workers (Schukken et al., 2014) to understand how currently available vaccine technologies might be applied as an adjunct to a comprehensive contagious mastitis pathogen control programme to mitigate spread of *S. aureus* between cows and possibly to reduce the incidence of heifer *S. aureus* IMI as demonstrated by Nickerson and co-authors (Nickerson et al., 1999). Such approaches must be evaluated in large-scale field trials, which to date are lacking. It will also be critical to define efficacy when conducting such studies. Definitions of efficacy will likely vary according to geographic location. In countries where clinical *S. aureus* mastitis is the major cost to the industry, vaccines that significantly reduce clinical disease may be defined as efficacious, whereas in countries where the major economic burden is through subclinical mastitis causing reduced milk yield and increasing SCC, prevention or early cure of IMI will define efficacy. Re-examining the utility of vaccine technologies to stimulate the immune response around the time of antibiotic treatment to increase treatment efficacy may also bear fruit. Overall, any vaccination strategy must be economically viable, particularly for the lower profit margin sector of the dairy industry such as sheep and goat production systems.

# 7 | CONCLUSION

## 7.1 | Major knowledge gaps

Staphylococcal mastitis is a complex disease. *Staphylococcus aureus* is a multifaceted pathogen that has the potential to express a myriad of virulence factors and is capable of evading immune surveillance and treatment compounds. These complexities are illustrated by the limited efficacy of currently available vaccines and anti-microbial treatments. To effectively combat this disease, a multifaceted approach must be taken. Control measures aimed at preventing *S. aureus* from entering the teat canal, namely milking time hygiene, have reduced the prevalence of this disease on many modern farms, yet the disease is still prevalent worldwide. In countries where dairying is a developing industry, it is likely that contagious mastitis caused by pathogens such as *S. aureus* and *Strep. agalactiae* may again become a prevalent disease due to lack of education or routine application of control measures. We need to understand better the complex interactions of *S. aureus* with dairy animals, and to fill knowledge gaps that are preventing us to devise more efficacious control measures. There are many knowledge gaps affecting progress on diagnosis, treatment and prophylaxis including vaccine development, from which it is difficult to extract a short-list. The following is an attempt based on current knowledge and interpretation of available data:

- Understanding of the genetic and clonal diversity of *S. aureus* strains infecting dairy ruminants and the molecular basis for pathogenesis of mastitis in relation to the antigenic variation of surface-presented and secreted proteins.
- Better knowledge of the (protective) immune response (cellular and humoral) including host transcriptomic analysis of *S. aureus* infection.
- Emphasis on basic research on cell-mediated immunity in the ruminant species and polarization of the immune response through the use of appropriate adjuvants.

## 7.2 | Priorities for research

The most important gaps could be bridged by:

- Searching the genetic arsenal of mastitis-causing strains to check whether the predominant clones share virulence factors which allow them to be successful parasites of the udder. Such work could be complemented by studies on the expression of these genes in the infectious setting.
- Developing experimental models to investigate the strategies used by *S. aureus* to survive within the mammary gland and resist treatments with anti-microbials: cell invasion, survival within phagocytes, biofilm or micro-colony formation, SCV, etc.
- Investigating the basis for cow-to-cow variation in response to *S. aureus* mastitis: genetics of pathogen-specific resistance/susceptibility/tolerance and influence of previous infection history (immune adaptive memory and innate imprinting).
- Identifying protective immune responses, both those responsible for the observed spontaneous cures and the vaccine-induced immune mechanisms.
- Investing in vaccine research and development to identify protective antigens that favour induction of protective immune responses including an examination of immunization schedules,

adjuvants and use of vaccines to augment intramammary therapy. Experimentation with small ruminant models is to be considered because of relevance to bovine mastitis and cost consideration.
- Investing in antibacterial discovery programmes to discover and develop new, more effective, narrow spectrum antibacterial agents for the treatment of *S. aureus* mastitis.
- Improved diagnostic methods (fast, cheap, sensitive and specific) to enable early detection and intervention through treatment or management.
- Incentive programmes for uptake and successful implementation of existing control measures.

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