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An update on environmental mastitis: Challenging perceptions

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Summary

Environmental mastitis is the most common and costly form of mastitis in modern dairy herds where contagious transmission of intramammary pathogens is controlled through implementation of standard mastitis prevention programmes. Environmental mastitis can be caused by a wide range of bacterial species, and binary classification of species as contagious or environmental is misleading, particularly for *Staphylococcus aureus*, *Streptococcus uberis* and other streptococcal species, including *Streptococcus agalactiae*. Bovine faeces, the indoor environment and used pasture are major sources of mastitis pathogens, including *Escherichia coli* and *S. uberis*. A faeco-oral transmission cycle may perpetuate and amplify the presence of such pathogens, including *Klebsiella pneumoniae* and *S. agalactiae*. Because of societal pressure to reduce reliance on antimicrobials as tools for mastitis control, management of environmental mastitis will increasingly need to be based on prevention. This requires a reduction in environmental exposure through bedding, pasture and pre-milking management and enhancement of the host response to bacterial challenge. Efficacious vaccines are available to reduce the impact of coliform mastitis, but vaccine development for gram-positive mastitis has not progressed beyond the "promising" stage for decades. Improved diagnostic tools to identify causative agents and transmission patterns may contribute to targeted use of antimicrobials and intervention measures. The most important tool for improved uptake of known mastitis prevention measures is communication. Development of better technical or biological tools for management of environmental mastitis must be accompanied by development of appropriate incentives and communication strategies for farmers and veterinarians, who may be confronted with government-mandated antimicrobial use targets if voluntary reduction is not implemented.

KEYWORDS
antimicrobial use, bedding, coliforms, environmental mastitis, molecular epidemiology, streptococci

1 | INTRODUCTION

The world population is growing and needs increasing amounts of food. We need food for more people, and we need more food per person as the global increase in average income drives changes in consumption patterns (Foresight, 2011). In 2007, Wen Jiabao, the then Premier of the People's Republic of China, said "I have a dream to provide every Chinese, especially children, sufficient milk each day." There are an estimated 1.4 billion people in China—more milk will be needed to satisfy Wen Jiabao's dream. At the same time, the growing world population puts increasing pressure on the availability of land and water. Land is needed for farming, for ecosystem services such as climate regulation, and for human habitation. To mitigate the risks of climate change, use of biofuels has been advocated.

This puts further pressure on the availability of land and water because biofuel production competes with feed and food production. To satisfy the many and conflicting demands on our planet, there is a clear need for sustainable intensification of food production, or "producing more with less" (Foresight, 2011). Reductions in waste, both before and after harvest, are a key component of sustainable food production. In dairy cattle, mastitis is a major cause of biological inefficiency or waste, for example, through lower yields, increased culling, discarded milk and impacts on fertility (Halasa et al., 2007; Seegers, Fourichon, & Beaudeau, 2003). In addition, mastitis affects animal welfare, which is highly valued in many industrialized countries (Byrd, Widmar, & Fulton, 2017; Tremetsberger, Leeb, & Winckler, 2015). Thus, there are many reasons to control mastitis in dairy cattle.

Mastitis, inflammation of the mammary gland, is primarily caused by bacterial intramammary infection (IMI). For control of bacterial infections in human and veterinary medicine, we often rely on the use of antimicrobials. Antimicrobial use (AMU) may contribute to antimicrobial resistance (AMR), which is another major societal concern relevant to milk production. The World Health Organization (WHO) recently endorsed a global action plan to tackle AMR and published a list of priority pathogens for research and development of new antibiotics (World Health Organization, 2015, 2017a). This list includes several mastitis pathogens, notably *Escherichia coli*, *Klebsiella* ("critical") and *Staphylococcus aureus* ("high priority"). They also produced a list of critically important antimicrobials for human medicine, which includes compounds that are used for mastitis treatment, for example, third- and fourth-generation cephalosporins (3/4GC) and fluoroquinolones ("critical") (World Health Organization, 2017b). Societal pressure is increasingly leading to calls for reduced AMU in animal agriculture, including dairy farming. In response to such pressures, quota or policies to reduce AMU are being proposed or implemented in several Western European countries (Dorado-García et al., 2016; O'Neill, 2016). Veterinarians and farmers will need to wean themselves from reliance on antimicrobials for mastitis control. Control of environmental mastitis without reliance on AMU depends on infection prevention, whereby host resistance, bacterial load and contact opportunities between hosts and pathogens are the key drivers of infection risk.

In the past few decades, dairy farming in the developed world has changed profoundly (Barkema et al., 2015). Concomitantly, there has been a major decrease in the prevalence of contagious mastitis and a relative or absolute increase in the incidence of environmental mastitis. In this study, we provide an overview of factors influencing the occurrence and control of environmental mastitis, which we define as mastitis caused by pathogens derived from the environment rather than from other infected cows in the herd. For many decades, the moniker "environmental mastitis" has been reserved for a limited number of species and genera, dominated by coliforms and *Streptococcus uberis*. We challenge this perception with data showing that many other pathogens, including *S. aureus* and *Streptococcus agalactiae*, can be environmental and argue that changes in host, pathogen and the environment, including societal and economic pressures, drive changes in the epidemiology and control of mastitis. Finally, we identify and prioritize gaps in our current knowledge of environmental mastitis, where further research or product development may be beneficial to the dairy industry, cattle health and human society.

2 | DISEASE IN THE NATURAL HOST

2.1 | Causative species and signs of environmental mastitis

Environmental mastitis is not a single disease but rather a disease syndrome with many potential causative agents and many contributing causes at host and environmental level. A brief description is given of infection- and host-response patterns for major gram-negative and gram-positive catalase-negative (GPCN) mastitis pathogens. Mastitis caused by *S. aureus* or *Mycoplasma* is described in detail in dedicated papers in this special issue, and mastitis caused by coagulase-negative staphylococci has recently been reviewed elsewhere (Vanderhaeghen et al., 2015). Algae of the genus *Prototheca* will not be covered, in part because it is not clear whether they are environmental or contagious pathogens (Jánosi et al., 2001; Osumi et al., 2008). In veterinary practice, there is often a perception that severe clinical mastitis (CM) (abnormalities in milk and mammary gland, accompanied by systemic signs) is primarily caused by coliform species, but severe CM may also be caused by streptococci (Figure 1) or *S. aureus* (Tassi et al., 2013; Zadoks et al., 2000). Conversely, mastitis caused by coliform species may be moderate (abnormalities in milk and mammary gland, no systemic signs), mild (abnormalities in milk only) or persistently subclinical (no visible signs) (Bradley & Green, 2000; Schukken et al., 2011a). Mild-to-moderate forms of clinical mastitis may also be caused by *S. agalactiae* (Barkema et al., 1998; Cortinhas et al., 2016). Thus, there is no one-to-one relationship between clinical severity and causative agent, nor is there a one-to-one relationship between mode of transmission and causative agent (see Section 3).

2.2 | Gram-negative mastitis

Mastitis caused by *E. coli* is generally transient and disease outcome largely depends on host factors, for example, lactation stage (Burvenich et al., 2003), energy balance (Suriyasathaporn et al., 2000), vitamin deficiency (Smith, Hogan, & Weiss, 1997) and vaccination status (Bradley et al., 2015a). Antibody-mediated immunity and neutrophil phagocytosis play a major role in the host response to *E. coli* mastitis, which may explain why vaccination against *E. coli* mastitis has been more successful than vaccination against other mastitis pathogens (Schukken et al., 2011b). Although most *E. coli* infections are transient, longitudinal studies with molecular typing of bacterial isolates have demonstrated that *E. coli* infections can be persistent, often with repeated episodes of CM linked by periods of subclinical infection (Döpfer et al., 1999). Subclinical coliform infections may start in the dry period and can manifest as CM in early lactation, up

FIGURE 1 Severe clinical mastitis characterized by abnormalities in milk (bottom right panel), mammary gland (left panel) and behaviour (top right panel) due to *Streptococcus uberis* infection. Photographs: RN Zadoks

to more than 100 days in milk (Bradley & Green, 2000). In herds with bulk milk somatic cell count (BMSCC) below 250,000 cells/ml, more than 50% of early lactation coliform CM were due to dry-period infection (Bradley & Green, 2000). The difference between onset of infection and clinical manifestation of disease has been attributed to polarization of the immune response and anti-inflammatory signalling during the dry period (Quesnell et al., 2012; Schukken et al., 2011b). Onset of infection in the dry period leading to CM in lactation has also been observed for *Klebsiella*, *Citrobacter* and *Serratia* spp. (Bradley & Green, 2000). For prevention of environmental mastitis, it is important to determine whether CM in early lactation is due to infections during the dry period or during lactation. Control measures need to target the relevant infection risks, for example, poor environmental hygiene or non-use of teat-sealants in the dry period, versus inadequate hygiene or nutrition in lactation.

The pathophysiology of IMI due to *Klebsiella*, *Enterobacter* spp. and non-coliform Enterobacteriaceae such as *Serratia* spp. is not as well studied as for *E. coli*, but there is a recent review dedicated to comparative analysis of their pathogenicity and immune response patterns (Schukken et al., 2012). In experimental studies, *Klebsiella* elicits more severe clinical signs and a stronger immune response than *E. coli*, whereby serum haptoglobin, interleukin (IL)-1 and IL-6 concentrations in serum are indicative of the chance of survival (Hisaeda et al., 2011). On-farm mortality due to *Klebsiella* can be high (Ostrum, Thomas, & Zadoks, 2008; Schukken et al., 2012). Bacteraemia may develop in cows with severe acute CM and contributes to mortality (Suojala, Kaartinen, & Pyörälä, 2013; Wenz et al., 2001). Bacteraemia may be caused by the mastitis pathogen or by bacteria from the gut or lung, for example, *Pasteurella* or *Salmonella* spp. (Wenz et al., 2001). Subclinical and mild clinical manifestations of *Klebsiella* mastitis also occur quite commonly (Oliveira, Hulland, & Ruegg, 2013; Figure 2). Knowledge of causative agents of CM can inform management decisions, for example, around vaccination or hygiene measures (see Section 5).
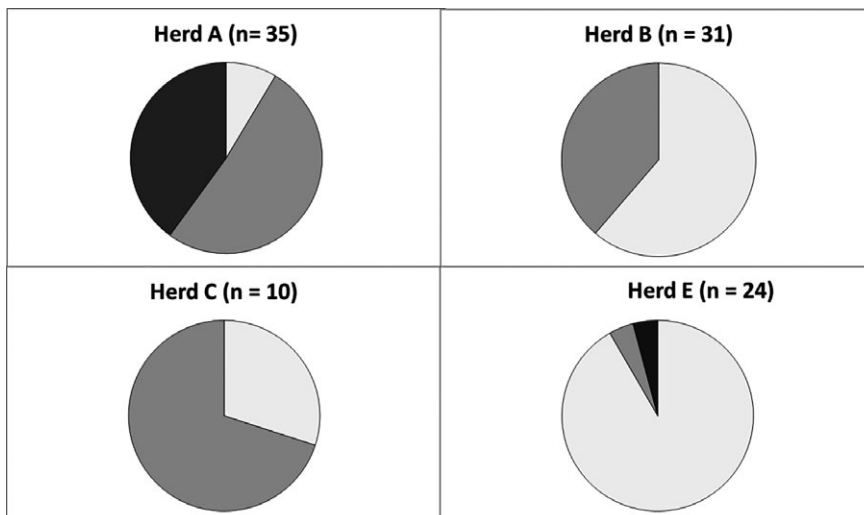
FIGURE 2 Herd-specific proportional distribution of mild-to-moderate cases of mastitis attributed to gram-negative pathogens. Black = *Enterobacter cloacae*; off-white = *Escherichia coli*; grey = *Klebsiella* spp. Number (*n*) of cases per herd shown in brackets. (Data: Schukken et al., 2011a; Herd D (*n* = 4) not shown)

Genomic analysis of mammary pathogenic *E. coli* (MPEC) suggests that the MPEC phenotype may have arisen from the wider *E. coli* population on multiple occasions (Richards et al., 2015). Isolates from both transient and persistent *E. coli* infections are genetically heterogeneous, and there is no consistent genotype or virulence profile associated with either manifestation, making the existence of an MPEC genotype a matter of debate (Dogan et al., 2012; Richards et al., 2015). Richards et al. (2015) noted that the type VI secretion system (T6SS) was present in all four MPEC isolates, compared with a prevalence of 38.6% in non-mammary isolates of *E. coli* (*n* = 56) and *Shigella* (*n* = 9), and suggested that further research should be conducted into the role of T6SS. This was not supported by comparative genomic analysis of *E. coli* by Kempf et al. (2016), who agreed with Dogan's conclusion regarding the absence of specific virulence genes. In phenotypic analysis of *E. coli* isolates, Kempf's colleagues identified the ability to resist phagocytosis and to ferment lactose as features associated with a mammary origin (Blum et al., 2008). Surprisingly, genes encoding lactose fermentation were not mentioned in the genomic studies on *E. coli* (Kempf et al., 2016; Richards et al., 2015).

For *Klebsiella*, as for *E. coli*, the ability to cause mastitis is not linked to any specific clade, but genomic analysis showed that genes associated with lactose fermentation were strongly overrepresented in isolates from mastitis (26 of 32) compared to those from bovine faeces (three of 19) or non-farm sources (Holt et al., 2015). This suggests that that mastitis-causing *Klebsiella*, like mastitis-causing *E. coli*, benefits from the ability to ferment lactose. The lactose operon was collocated with an iron-enterobactin operon. Thirty bovine isolates carrying both operons were found in 23 different lineages of *K. pneumoniae* phylogroups I and II, demonstrating that they are linked and subject to extensive horizontal transfer (Holt et al., 2015). Interestingly, the ferric enterobactin receptor FepA was an early target for development of a *Klebsiella* vaccine (Lin, Hogan, & Smith, 1999). In dry cow secretion, antibodies against FepA inhibited the growth of all *E. coli* isolates but less than half of *K. pneumoniae* isolates (43%) (Lin et al., 1999). This observation might be explained, in part, by the fact that the enterobactin gene is chromosomally located in *E. coli* but largely plasmidborne and hence less consistently present in *Klebsiella* (Holt et al., 2015; Kempf et al., 2016). Alternative vaccine targets for *Klebsiella* mastitis are yet to be identified.

2.3 | Gram-positive catalase-negative cocci

In many studies and diagnostic laboratories, all GPCN other than *S. agalactiae* are lumped under the badge "environmental streptococci" or "*Streptococcus* spp." (Cameron et al., 2016; Oliveira et al., 2013). Both misnomers cover streptococci, enterococci and lactococci, among others. The major streptococci are *S. uberis* and *Streptococcus dysgalactiae*, the major enterococci are *Enterococcus faecium* and *Enterococcus faecalis*, and the main lactococci are *Lactococcus lactis* and *Lactococcus garvieae* (Cameron et al., 2016; Petersson-Wolfe, Wolf, & Hogan, 2009). The role of enterococci as causative

agents of mastitis has long been recognized whereas *Lactococcus* spp., previously studied as potential tools in mastitis prevention or treatment, have only recently been recognized as mastitis pathogens in their own right (Plumed-Ferrer et al., 2013; Rodrigues et al., 2016). The advent of advanced diagnostic methods has aided the recognition of GPCN species. With the exception of *S. uberis*, however, relatively little is known about shedding patterns, pathogenesis and host immune response to those pathogens. A PubMed search of "[pathogen name] mastitis challenge" yielded 58, 13, 2 and 5 hits for *S. uberis*, *S. dysgalactiae*, *Enterococcus* and *Lactococcus*, respectively, with more than 20 experimental challenge studies for *S. uberis*, and none or very few for the other species or genera. This reveals a surprising knowledge gap for *S. dysgalactiae*. Although its status as environmental versus contagious pathogen may be debated (Fox & Gay, 1993; Smith, Todhunter, & Schoenberger, 1985), its importance as mastitis pathogen is beyond doubt, often on a par with or even exceeding the prevalence or incidence of *S. uberis* (Lundberg et al., 2014; Sampimon et al., 2009a).

Within *S. uberis*, multiple clonal complexes (CC) are recognized and virulence is higher for CC5, which is largely associated with CM, than for CC143, which is predominantly associated with subclinical mastitis, or CC86, which has been linked to latent infection (Tomita et al., 2008). Strain-specific virulence can be replicated in vivo (Hill, 1988; Tassi et al., 2013) and is associated with differences in uptake and killing by neutrophils or monocytes in vitro (Hill, 1988; Tassi et al., 2015). There is, however, no obvious association between outcome of infection and gene content (Hossain et al., 2015). Strain-specific virulence has also been documented for *E. faecium* (Petersson-Wolfe et al., 2009). Potential virulence genes underpinning such differences have not been studied in enterococci, but the genomic tools that have been developed to study virulence factors of *S. uberis* mastitis could provide insight into the functional genomics of other GPCN species (Blanchard et al., 2016). As for coliforms, host characteristics affect the outcome of GPCN infections: cows in early lactation responded differently to *E. faecium* challenge than those in late lactation, and a *S. uberis* strain that largely failed to cause infection in mid-lactation animals had been isolated from CM at parturition (Petersson-Wolfe et al., 2009; Tassi et al., 2013). In vitro, macrophages from dry cow secretion are more active against *S. uberis* than those from mid-lactation cows, even though *S. uberis* infections commonly occur in the dry period (Denis et al., 2006). The role of mammary epithelium in pathogenesis of *S. uberis* mastitis is debated and has variously been described as linked to infection outcome (Tassi et al., 2015), largely irrelevant (Leigh, 1999) or sufficiently common and critical to base vaccine development around it (Almeida et al., 2015; see Vaccines).

Streptococcus agalactiae is currently not considered as one of the "environmental streptococci," but we argue that this GPCN species may be of environmental origin with humans acting as reservoir. Challenge studies comparing the bovine host response to *S. agalactiae* of human and bovine origin were published in the early 1980s and are poorly known in the current mastitis community (Jensen, 1982; Van den Heever & Giesecke, 1980). Inoculation of bovine

mammary glands with *S. agalactiae* from humans, where it is primarily known as group B Streptococcus, results in an acute response characterized by CM with milk losses greater than those observed after challenge with bovine strains (Jensen, 1982; Van den Heever & Giesecke, 1980). Infections with human strains are more likely to cure spontaneously than those caused by bovine strain (Jensen, 1982). This, combined with lower levels of bacterial shedding, limits the opportunity for contagious transmission (Jensen, 1982). Strain-specific shedding has also been documented in field studies (Mahmmod et al., 2015). The observations from experimental challenge studies may explain why CM due to *S. agalactiae* is occasionally observed in low BMSCC herds without apparent within-herd transmission (Barkema et al., 1998). The authors are aware of similar anecdotal reports from large dairy herds in the United States, where AMR was described as an additional feature of such uncharacteristic clinical and epidemiological manifestations of *S. agalactiae*. Strain typing studies support the occasional occurrence of human-derived strains in dairy cattle, including the presence of AMR determinants that are typical of human as opposed to bovine *S. agalactiae* (Dogan et al., 2005).

2.4 | Porte d'entree

For most mastitis pathogens, the teat end is considered the porte d'entree into the mammary gland. It has been suggested that presence of minor pathogens (non-aureus staphylococci, corynebacteria) at the teat end may protect against infection with major pathogens (Reyher et al., 2012). The authors of a recent review (Reyher et al., 2012) concluded that observational studies showed no such effect, "whereas challenge studies showed strong and significant protective effects, specifically when major pathogens were introduced into the mammary gland via methods bypassing the teat end." Physical or physicochemical characteristics of the teat end may contribute to that discrepancy, such as the amount of keratin present, peak flow rate and teat canal length (Capuco et al., 1992; Lacy-Hulbert & Hillerton, 1995). In some modern large herds, for example, in the High Plains area of the western United States, milk production is measured per hour rather than per cow, acre or person. The emphasis on milking speed may potentially contribute to teat-duct patency and increased risk of environmental mastitis. This could be a factor contributing to the high incidence of *Klebsiella* mastitis in the United States compared to Europe. There is almost no evidence on the role of flow rate and teat-end characteristics in susceptibility to gram-negative mastitis. Even less is known about the role of the teat-end microbiota. Teat-end microbiota differ between healthy quarters with or without a history of mastitis (Falentin et al., 2016). Quarters without a history of CM had higher microbial diversity, more members of the class Clostridia, the phylum Bacteroidetes and the order Bifidobacteriales, and fewer members of the classes Bacilli, which includes staphylococci, and Chlamydia. Whether such differences are a cause or consequence of CM or antimicrobial treatment is unknown (Falentin et al., 2016). Further research into the composition and role of teat-end microbiota, the impact of teat disinfectants and antimicrobial treatment, and potential microbiota manipulations may provide new insight or tools for environmental mastitis control.

3 | EPIDEMIOLOGY

3.1 | Pathogen characteristics

Molecular epidemiology studies have been important in elucidating the range of transmission modes within mastitis-causing pathogen species, and it is increasingly clear that the distinction between contagious and environmental pathogens should be applied at strain level rather than species level (Gurjar et al., 2012; Zadoks et al., 2011a). *Streptococcus agalactiae*, long considered the quintessential contagious pathogen, may originate from humans (Dogan et al., 2005) or faeces (Farre et al., 2017; Jørgensen et al., 2016). *Klebsiella pneumoniae*, almost exclusively seen as environmental pathogen, may occasionally spread from cow to cow (Munoz et al., 2007; Schukken et al., 2011a). In human medicine, there is increasing recognition that most people carry *S. aureus* and that patients may become infected with their own strain of the pathogen whilst staying in the same hospital (Price et al., 2017). Likewise, cows staying on the same dairy farm may become infected with their own individual or environmental strains of *S. aureus* (Zadoks et al., 2011a). Control strategies that reduce contagious transmission do not affect the occurrence of environmental *S. aureus* mastitis (Sommerhäuser et al., 2003). The possibility of contagious transmission of *S. uberis* was demonstrated with molecular tools more than a decade ago, and it is now acknowledged that cow-to-cow transmission may be the predominant route of infection in many dairy herds (Davies et al., 2016; Zadoks et al., 2003). Veterinarians' and researchers' insistence on erroneously classifying *S. aureus* as contagious pathogen and *S. uberis* as environmental pathogen leads to false emphasis on mastitis control methods that may be irrelevant to a farm's situation. For example, a major overhaul of the parlour routine will not resolve an environmental *S. aureus* mastitis problem (Gurjar et al., 2012).

Although strain typing has been used in numerous mastitis studies, there is some confusion around the epidemiological interpretation of such data. Strain heterogeneity is often interpreted as evidence of environmental mastitis, and strain homogeneity is interpreted as evidence of contagious transmission. The former is correct, but the latter is not (Figure 3). Strain homogeneity can result from contagious transmission or environmental point source infection, as shown for mastitis outbreaks caused by *Pseudomonas aeruginosa* (Daly et al., 1999) and *Serratia* spp. (Muellner et al., 2011). Additional epidemiological investigation and testing of environmental samples can be used to place molecular data in context (Muellner et al., 2011; Munoz et al., 2007). Few diagnostic laboratories offer strain typing as a routine method to differentiate between potential epidemiological scenarios within a herd. When offered, strain typing is currently based on comparative analysis of multiple isolates from a single herd (Gurjar et al., 2012). To date, there are no definitive methods to identify a single coliform, streptococcal or staphylococcal isolate as environmental opportunist or potentially contagious

FIGURE 3 Modes of transmission (left: contagious; centre: environmental point source; right: heterogeneous environmental source) and resultant patterns of strain distribution (left, centre: homogeneous; right: heterogeneous), demonstrating that strain heterogeneity is proof of environmental origin of mastitis pathogens, but homogeneity is not proof of contagious transmission
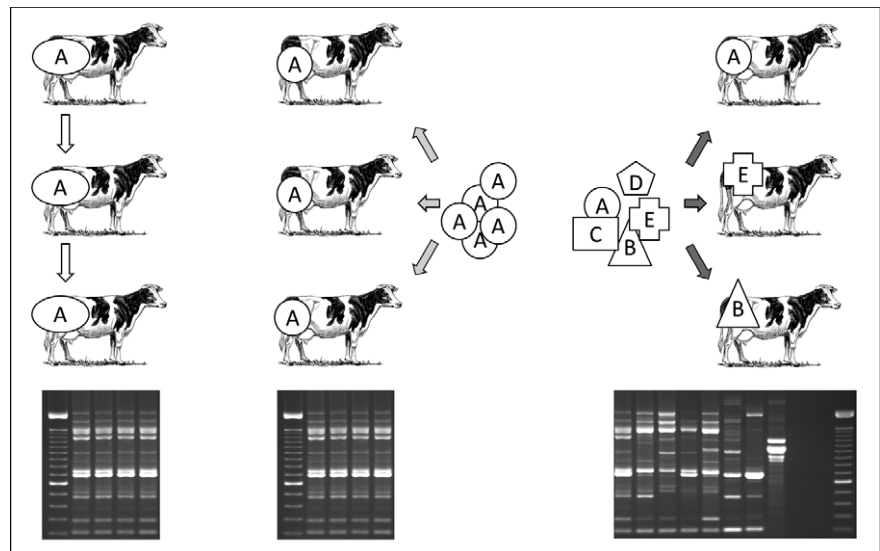
pathogen. For gram-positive mastitis pathogens, there is some evidence that transmission may be a function of the pathogen, as observed rates of transmission differ between strains that are present in the same herd (Smith, Fox, & Middleton, 1998; Zadoks et al., 2003). Host factors such as shedding level or milk leakage may also affect transmission, as do environmental and herd management factors, including bedding hygiene and teat disinfection (Munoz et al., 2007; Zadoks et al., 2001). Routine availability of strain typing as a diagnostic tool and recognition of the non-binary nature of mastitis pathogens could contribute to improved mastitis control.

3.2 | Host range

Most major mastitis pathogens are not host-specific. *Streptococcus agalactiae*, often erroneously described as an "obligate intramammary pathogen," is a commensal in humans, with 20%–40% of healthy men and women carrying the organism in their urogenital tract, gastrointestinal tract or throat (reviewed in Lyhs et al., 2016). Several strands of indirect evidence suggest that milkers may introduce the pathogen into cattle herds (reviewed in Lyhs et al., 2016). It also affects fishes and can be found in marine, fresh and waste water (reviewed in Delannoy et al., 2013). Within the species *Streptococcus dysgalactiae*, two subspecies are recognized, that is, *S. dysgalactiae* subsp. *equisimilis* and *S. dysgalactiae* subsp. *dysgalactiae*. The former is a commensal and pathogen of people but rarely affects cattle. The latter is a frequent mastitis pathogen and commonly referred to as *S. dysgalactiae* in the veterinary literature (Lundberg et al., 2014). In sheep, *S. dysgalactiae* subsp. *dysgalactiae* causes polyarthritis or joint ill in lambs, but it rarely causes mastitis. *S. uberis* and *E. coli* are also common mastitis pathogens in cattle but relatively rare in sheep (Gelasakis et al., 2015; Zadoks et al., 2014). Conversely, *Mannheimia haemolytica* mastitis is common in sheep but not in cattle, whereas *S. aureus* is common in both host species (Gelasakis et al., 2015; Zadoks et al., 2011a) and also in people (Price et al., 2017; Zadoks et al., 2014). The mechanisms underpinning observed differences in

host preference are poorly studied and may provide insights into host-adaptation or virulence factors. Pigs, dogs and cats may occasionally act as sources of mastitis pathogens, with pigs playing a role in MRSA transmission (see Socio-economic aspects), and dogs or cats acting as a source of *S. canis* (Richards et al., 2012).

3.3 | Vectors

Mastitis pathogens are rarely vector-transmitted. Insect vectors such as flies and wasps may play a role in transmission of some mastitis pathogens, notably *S. aureus*, *S. dysgalactiae* and pathogens associated with summer mastitis (Chirico et al., 1997; Yeruham, Schwimmer, & Brami, 2002). Vectorborne mastitis may affect non-lactating cattle but should probably be classed as contagious mastitis because pathogens are transmitted from host to host by the vectors (Owens et al., 1998). A role of stable flies in transmission of *E. coli* mastitis has been suggested but not proven (Castro et al., 2016). Environmental controls, that is, insect control, may reduce the risk of vectorborne mastitis, but an investigation of the impact of fly control in heifers on early lactation CM yielded results that depended on selection of the outcome variable of interest (Green et al., 2007).

3.4 | Reservoirs

The major reservoirs for environmental pathogens are unused or used bedding material and bovine faeces. For example, sawdust is a recognized risk factor for *Klebsiella* mastitis (Ericsson Unnerstad et al., 2009; Munoz et al., 2007). Composted bedded pack (CBP) systems and peat have recently been associated with outbreaks of *K. pneumoniae* mastitis in Denmark. In those outbreaks, it is not known whether bedding served as the original source of the pathogen or merely as growth medium for its amplification. Peat and straw bedding are both recognized as risk factors for *S. uberis* mastitis (Ericsson Unnerstad et al., 2009), but *S. uberis* is also highly prevalent during the pasture season in the Netherlands and in the

pasture-based system of New Zealand (Lopez-Benavides et al., 2007; Olde Riekerink, Barkema, & Stryhn, 2007). Due to high cost and lack of availability of traditional bedding materials, the use of physically separated slurry or recycled manure solids (RMS) as bedding material has grown in recent years. RMS may be obtained through separation of anaerobic digested manure, separation of raw manure or separation of raw manure followed by mechanical drum-composting (Husfeldt et al., 2012; Leach et al., 2015). Drum-composted manure solids contained no coliform bacteria prior to use as bedding, in contrast to digested and raw manure (Husfeldt et al., 2012). Even if composted solids contain no coliforms prior to use, they are a rich source of nutrition for bacteria, and once used, there is no difference in coliform counts between composted, digested and raw manure (Husfeldt et al., 2012). Control methods may differ between categories of pathogens, both for RMS (Leach et al., 2015; Rowbotham & Ruegg, 2016) and for CBP (Eckelkamp et al., 2016). Leach et al. (2015) warn that caution is needed when adopting RMS use in Europe under climatic conditions that differ from the dry climates in the United States where its use was developed. Moreover, they warn that little is known about the impact of RMS use on AMR (Leach et al., 2015). Dairy farm slurry can be a source of resistant pathogens. For example, ESBL-resistant *E. coli* was detected in slurry from 41% of herds in a study in the Netherlands (Gonggrijp et al., 2016). With growing concern about AMR (see Socio-Economic aspects), better understanding of the impact of manure recycling on both udder health and AMR is needed. Use of inorganic bedding, for example, sand, is recommended to reduce the environmental load of opportunistic pathogens, but high loads of *E. coli*, *Klebsiella* and GPCN cocci have been found in sand bedding (Kristula et al., 2005; Munoz et al., 2006). High bacterial counts can result from on-farm recycling of sand or poor bedding management. Once mixed with manure, any type of bedding becomes a source of pathogens and the use of sand may give a false sense of security, leading to poor maintenance of stalls (Figure 4). Barn conditions rather than bedding type may be the main determinants of bacterial counts (Zehner et al., 1986).

Faecal shedding has been documented for *S. agalactiae* (Jørgensen et al., 2016), *S. uberis* (Zadoks, Tikofsky, & Boor, 2005) and *Klebsiella* (Munoz et al., 2006), and average faecal prevalence ranges from 5% to 23% and >80%, respectively, with considerable

differences between farms. The faecal prevalence of *S. aureus* in cattle ranges from 1.4% to 12%, based on testing of faecal swabs (Dimitracopoulos, Kalkani-Boussiakou, & Papavassiliou, 1977; Roberson et al., 1994). Faecal contamination turns not just bedding but also alleys, traffic lanes, water troughs and the outdoor environment into sources of environmental pathogens. *S. agalactiae* has been found in milking parlours, alleys, stalls and water troughs (Jørgensen et al., 2016). *Streptococcus uberis* can be found in bedding, traffic lanes, water troughs and the outdoor environment, including soil and grass (Lopez-Benavides et al., 2007; Zadoks et al., 2005). In the absence of cattle, *S. uberis* is undetectable, or levels decline rapidly, implying that cattle, and most likely cattle faeces, are the original source of environmental *S. uberis* (Lopez-Benavides et al., 2007; Zadoks et al., 2005). The prevalence of *Klebsiella* in beds, alleys, on legs and on teats is very similar to the level of faecal shedding in the same herd, whilst a higher prevalence was detected in drinking water and a lower prevalence in feed (Zadoks et al., 2011b). The presence of gram-negative and gram-positive organisms in faeces and drinking water suggests that an oro-faecal transmission cycle exists for several major mastitis pathogens, including *S. agalactiae* and *K. pneumoniae* (Jørgensen et al., 2016; Zadoks et al., 2011b). With the exception of *S. agalactiae*, there was considerable strain heterogeneity within environmental sources of pathogens, which can be attributed to between- and within-animal heterogeneity of strains in faeces (Munoz & Zadoks, 2007; Zadoks et al., 2005). The faecal bacterial load in the environment is a function of initial contamination, subsequent amplification and removal and can be managed to reduce the challenge to the cows' immune system (see Section 5).

4 | SOCIO-ECONOMIC IMPACT AND ZOONOTIC ASPECTS

In addition to societal pressures outlined in the Introduction, there are financial pressures on dairy farming. When supermarkets charge more for soft drinks, which are essentially bottles of water with additives, than for a bottle of milk produced by sentient beings, the financial pressures on dairy production become visible: not only do we need to produce "more with less" in terms of physical resources, we also need to produce "more with less" in terms of financial and human resources (Figure 5). Non-antimicrobial control of bacterial IMI is both labour-intensive and knowledge–intensive, and the shortage of appropriately trained staff is an increasing problem (Maloney, 2002; Tipples & Trafford, 2011). In some countries, expensive labour is replaced by automation, for example, of milking machines or alley scrapers, whilst dairy care and milk harvesting rely heavily on indigenous or foreign human labour in other countries (Barkema et al., 2015).

Direct and indirect economic losses due to mastitis have been estimated (Halasa et al., 2007) and vary greatly between animals and pathogens. For example, yield losses in heifers are greatest after *E. coli* CM and in multiparous animals after *Klebsiella* CM, and both coliforms have greater impact on fertility than other pathogen

FIGURE 4 Faecal contamination is a major source of exposure to environmental pathogens regardless of the use of sawdust (left), straw (right) or other bedding material. Photographs: RN Zadoks

FIGURE 5 "Amazing value milk," produced by sentient beings but sold at a lower price than soft drinks, illustrating financial pressures on the dairy industry. If the soft drinks had not been on sale, they would have cost more than twice as much as milk. Photographs: RN Zadoks

species (Hertl et al., 2014a, 2014b). Yield losses may persist for months after coliform or GPCN mastitis, whilst CM with non-aureus staphylococci does not cause reduced production (Hertl et al., 2014a). The association of pathogens with culling risk differs between heifers and multiparous animals, lactation stages and number of CM episodes, with different combinations of factors identifying different pathogen species as being associated with the highest risk of culling (Cha et al., 2013; Gröhn et al., 2005). Other costs of mastitis are even harder to quantify and relate to its impact on public perception, notably perceptions around animal welfare and use of antimicrobials. This creates a dilemma as treatment of mastitis may be necessary for welfare reasons, and would often involve the use of antimicrobials.

Of major concern from a zoonotic perspective are methicillin-resistant *S. aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL)-producing coliforms. Studies based on data collected around the millennium (1994–2001) showed little evidence for a relationship between use of antimicrobials for mastitis control and AMR (Erskine et al., 2002; Makovec & Ruegg, 2003). In the early 21st century, however, we have seen the emergence of MRSA in cattle in Europe and elsewhere. Molecular evidence suggests that some MRSA, notably MRSA carrying the *mecC* gene rather than the more common *mecA* gene, may have arisen in cattle, whereas *mecA* MRSA probably originates in other host species (Holmes & Zadoks, 2011). MRSA was first recognized as a cause of mastitis in dairy cattle in Belgium where it was thought to originate from people (Devriese & Hommez, 1975). Currently, most MRSA of dairy origin in Belgium and several other European countries belong to sequence type (ST) 398, which is highly prevalent in pigs (Locatelli et al., 2016; Vanderhaeghen et al., 2010). Transmission from people or pigs, both of which are environmental sources from the mammary gland's perspective, is likely to explain introduction into dairy herds. As for *S. canis*, initial introduction from an external source may be followed by within-herd contagious transmission (Tavakol et al., 2012; Vanderhaeghen et al., 2010). Pig and pig farm numbers are correlated with the risk of MRSA detection in bulk milk (Locatelli et al., 2016). Proximity to pig farms was also identified as risk factor for detection of ESBL *E. coli* in organic dairy farms, albeit based on slurry samples rather than milk samples from cows with mastitis (Santman-Berends et al., 2017). Those examples show that the environment within the farm and beyond the farm may contribute to occurrence of mastitis and AMR.

ESBL-producing coliforms are rarely identified in bovine mastitis in Europe. In France and Italy, 0.4% of 1,427 mastitis-derived *E. coli* and

Klebsiella isolates and 0.7% of 140 *Klebsiella* isolates, respectively, were ESBL-positive (Dahmen et al., 2013; Locatelli et al., 2010). Similarly, in Canada, ESBL was not detected among 394 *E. coli* and 139 *Klebsiella* isolates from bovine milk (Saini et al., 2012). By contrast, in China, almost a quarter of *E. coli* isolates from bovine mastitis were ESBL-producing coliforms (Ali et al., 2016). In the UK and the Netherlands, presence of ESBL-coliforms has been linked to presence or use of 3/4GC in waste milk and slurry (Gonggrijp et al., 2016; Randall et al., 2014). Despite the low prevalence of ESBL-producers among mastitis pathogens in Western countries, the association between 3/4GC use and ESBL prevalence on dairy farms together with WHO concerns about use of those compounds in animals will in all likelihood limit their availability as mastitis treatment products. In the United States, extra-label use of 3/4GC was banned (Federal Drug Administration, 2012). Considering that cephalosporins and fluoroquinolones are the only compounds with some evidence for beneficial effects in treatment of coliform mastitis (Schukken et al., 2011a; Suojala et al., 2013), restrictions on their use make prevention of environmental mastitis even more important.

5 | PREVENTION, DETECTION AND CONTROL

5.1 | Biosecurity

External biosecurity, that is, prevention of introduction of pathogens into the herd, is of limited effect for environmental mastitis because most environmental mastitis pathogens are part of the normal faecal flora of dairy cows. Bedding materials and healthcare products may be a source of pathogens, as described before for *Klebsiella* in sawdust and *Pasteurella* in teat wipes. Presence of pathogens in a healthcare product does not necessarily indicate that this product was an external source of pathogens. In a cluster of *Serratia* outbreaks, farm-specific strains of the pathogen were identified, and the outbreaks were associated with unhygienic handling of teat-dip, resulting in contamination with *Serratia* and subsequent growth (Muellner et al., 2011). In Denmark, movement of cattle from *S. agalactiae*-positive herds was not allowed until 2005 for reasons of external biosecurity. There was no association, however, between animal movements and a change to *S. agalactiae*-positive herd status (Mweu et al., 2012, 2014). This supports the notion that *S. agalactiae* may be derived from non-bovine, that is, environmental, reservoirs.

Measures to reduce bacterial exposure can be taken in the milking parlour and elsewhere. In Europe, use of pre-dips containing disinfectants to reduce bacterial load prior to milking is rare or even prohibited, whilst its use is common in the United States. Regardless of whether a wet (pre-dip used) or dry (no pre-dip used) pre-milking routine is adopted, it is important to evaluate the effect of the procedure. Scoring tools for cow, udder and teat cleanliness have been developed to assist with this task, and their use has demonstrated an association between dirty udders and risk of new infection (Dohmen, Neijenhuis, & Hogeveen, 2010) or high bacterial counts on teats (Guarín, Baumberger, & Ruegg, 2017; Munoz et al., 2008). Moreover, it has been shown that the efficacy of pre-milking teat disinfection is lower for dirty teats than for clean teats (Munoz et al., 2008; Zdanowicz et al., 2004). Scoring systems have also been developed for teat-end callosity or hyperkeratosis (Shearn & Hillerton, 1996). In a small study (135 cows), teat-end hyperkeratosis was not associated with the risk of mastitis, but a large study (1,667 cows) showed that severe hyperkeratosis is associated with increased risk of *E. coli* or *S. uberis* CM, and moderate hyperkeratosis with increased risk of *E. coli* CM (Breen, Green, & Bradley, 2009; Zoche-Golob et al., 2015). Bacterial loads of both organisms are higher in teat ends with hyperkeratosis than in those without, providing a plausible biological mechanism for the observed association (Paduch, Mohr, & Krömker, 2012). Hyperkeratosis is associated with the duration of milking, and particularly with overmilking, which may be an issue in large parlours if automated cluster removal is not used or settings are incorrect (Edwards et al., 2013). Thus, although milking machine settings and parlour routines are primarily associated with contagious mastitis, they do also impact on the risk of environmental mastitis. With the introduction of automated milking systems (AMS), the milking frequency is increased, particularly for high-yielding cows. This may be beneficial, through frequent removal of bacteria and replenishment of somatic cells in the mammary gland, or harmful due to frequent opening of the teat canal. Milk leakage is more common in cows milked by AMS than in a milking parlour, particularly for cows with high milk flow (Klaas et al., 2005; Persson Waller et al., 2003). This could lead to higher risk for the cow itself, or for other cows in the herd if the cow leaks milk with high bacterial loads (Munoz et al., 2007). Although consensus on udder health benefits may not exist, AMS are gaining ground.

Tools to manage bacterial counts in bedding include bedding replacement and the use of bedding conditioners. Both alkaline and acidic conditioners have been used successfully to modify coliform and streptococcal counts on cow mattresses (Kristula et al., 2008) and in sawdust (Paduch, Mohr, & Krömker, 2013; Proietto et al., 2013). When using lime, both positive and negative effects were observed at teat level, that is, a reduction of bacterial counts as well as damage to teat skin (Kristula et al., 2008; Paduch et al., 2013). With acidifiers, neither positive nor negative effects were observed (Kristula et al., 2008; Proietto et al., 2013). The effect of acidifiers is time-limited. In comparison with untreated control bedding, bacterial counts in treated sawdust or recycled manure were reduced on day 1 after addition, but not on day 2 or day 6, suggesting that daily addition of conditioner may be needed to maintain reduced bacterial counts (Hogan, Wolf, & Petersson-Wolfe, 2007). When RMS are used as bedding material, either as top layer on mattresses or as deep layer, daily replacement reduces coliform counts, specifically for *Klebsiella*, but the same management strategy increased streptococcal counts (Sorter, Kester, & Hogan, 2014). In CBP, coliform and streptococcal counts differ from each other in their association with cow density, ambient or internal temperature and carbon:nitrogen ratio (Black et al., 2014; Eckelkamp et al., 2016). There is no single optimal method to choose or manage bedding to reduce exposure to all types of environmental pathogens (Leach et al., 2015; Rowbotham & Ruegg, 2016).

Infection risk in dry cows is predominantly driven by herd and management rather than cow factors (Bradley et al., 2015b; Green et al., 2007). Green et al. (2007) grouped risk factors, which include protective factors, by stage of the dry period, that is, the drying-off process itself, early dry period, late dry period or transition period, and finally the calving period. For cows that were housed during the dry period, protective effects were observed for good drainage in the early dry-cow cubicle accommodation, use of mattresses on dry-cow and transition-cow cubicle surfaces, disinfection of cubicle bedding for the early dry period or the close-up groups, scraping of the feed and loaf area at least once daily, and bedding of cubicles at least once daily. Dry cows housed in straw yards, where disinfection is not an option, and transition cows that were housed with milking cows were at increased risk of CM in early lactation (Green et al., 2007). Although this analysis was based on detection of CM in early lactation rather than on detection of IMI during the dry period, the association between dry-period IMI and lactational CM (see Section 2) suggests that prevention of dry-period IMI through reduced exposure to pathogens explains the observations, at least in part. For cows that were out on pasture during the dry period, a pasture rotation method of 2 weeks of grazing by dry cows followed by 4 weeks without grazing reduced the risk of early lactation CM (Green et al., 2007). This may be due to a reduction in bacterial load on pasture in the absence of cattle, as demonstrated for *S. uberis* (see Section 3.4). Specific advice on straw yard management, which was used to house more than half of the cattle in the study, could not be derived from the data (Green et al., 2007).

5.2 | Detection

Diagnostics can be used to detect mastitis, that is, mammary gland inflammation, or IMI, that is, pathogen presence. Inflammation can be detected based on somatic cell count (SCC), electrolytes, enzymatic markers or acute phase proteins (Pyörälä et al., 2011; Viguier et al., 2009). As methods for pathogen detection become more sensitive, the ability to differentiate between pathogen-positive samples with and without evidence of inflammation becomes increasingly important, particularly when testing is conducted to inform treatment decisions, bearing in mind the societal pressure to reduce AMU. Detection of IMI has traditionally been based on culture, but there is a wide range of opinions on how to interpret culture results

(Dohoo et al., 2011). Species identity of cultured bacteria can be confirmed with phenotypic or genotypic methods (Zadoks & Watts, 2009). Phenotypic identification using biochemical profiles is unreliable for many mastitis pathogens, including *Klebsiella* and *Staphylococcus* spp. (Munoz et al., 2007; Sampimon et al., 2009b). Modern phenotypic testing is increasingly based on proteomics, notably matrix-assisted laser desorption ionization time-of-flight mass spectrometry analysis (Cameron et al., 2017; Schabauer et al., 2014). Its application directly to milk samples may be possible but only at high bacterial concentrations (Barreiro et al., 2017). PCR or sequencing of housekeeping genes for species detection or identification is commonly applied to milk samples (PCR) and cultured isolates (sequencing), respectively, although both methods can be used for both sample types. PCR-based detection of mastitis pathogens in milk has been used commercially for almost a decade (Koskinen et al., 2009) and is very popular in Europe's Nordic countries whilst uptake is slower elsewhere. PCR panels targeting few or many pathogens are available, and pathogens may be detected at species or genus level. Detection of *blaZ*, encoding penicillin resistance, is also possible, but PCR may not be sufficient to determine whether a staphylococcal resistance gene is present in *S. aureus* or other staphylococci (Koskinen et al., 2009; Virgin et al., 2009). For PCR, as for culture, interpretation of results is subject to debate. The increased sensitivity of PCR, which detects bacteria that are non-viable, viable but difficult to culture or easy to culture, is an advantage over culture, which only detects the last category. However, increased sensitivity may be accompanied by decreased specificity, for example, positive PCR results due to sample contamination (Koskinen et al., 2010). Moreover, work on milk microbiota has shown that several mastitis-causing organisms are commonly detected in healthy mammary glands (Oikonomou et al., 2012). The microbiota is the totality of bacterial species present based on culture-independent analysis of 16S rDNA sequences. Its composition and role in the mammary gland have recently been reviewed (Addis et al., 2016). Crucially, microbiota studies suggest that mastitis should probably not be attributed to intramammary infection of a normally sterile organ but to dysbiosis in a gland that has a highly diverse microbiota when it is healthy. Further insight into milk microbiota may contribute to new mastitis control tools.

A key component of discussions about diagnostics is their intended use. Satisfactory results in decision-making around targeted versus blanket antimicrobial DCT have been made using records of SCC and CM, without knowledge of pathogen presence or microbiota composition (Scherpenzeel et al., 2016). In this situation, the cost of pathogen detection is unlikely to be justifiable. By contrast, on-farm culture to inform treatment decisions for lactational CM has recently gained popularity because it allows for a reduction in diagnostic turnaround time and antimicrobial use (Lago et al., 2011a; Mansion-de Vries et al., 2014). Treatment decisions, and hence the utility of diagnostics, hinge on treatment options, which may change over time. Currently, they are predicated on the premise that antimicrobial treatment is justified for gram-positive mastitis but not for culture-negative mastitis or mild-to-moderate gram-negative mastitis

(see Section 5.3). Possibly of greater importance for test uptake is farmer perception of what constitutes a useful test. In the Netherlands, 34% of farmers submit milk samples from CM to a diagnostic laboratory, whereas 71% would consider use of an on-farm test, depending on the time-to-result (Griffioen et al., 2016). A relatively novel mastitis-diagnostic with potential for short time-to-result is loop-mediated isothermal amplification (LAMP). LAMP primers are available for *S. aureus* (Sheet et al., 2016) and major streptococci (Bosward et al., 2016; Wang & Liu, 2015), and implementation as pregnancy-test-like lateral flow device is possible (Cornelissen et al., 2016). A major challenge for on-farm molecular detection of pathogens or AMR genes is the large number of bacterial species and resistance genes that may be present in milk or mastitis pathogens. There is a single penicillin-resistance gene in *S. aureus*, which is covered by commercially available PCR, but there are multiple categories of ESBL genes in coliforms (bla_{SHV}, bla_{TEM} and bla_{CTX-M} ESBL), with multiple clusters of bla_{CTX-M} genes (e.g., $bla_{CTX-M-1}$, $bla_{CTX-M-2}$ and $bla_{CTX-M-9}$) and multiple genes within each cluster (Trang et al., 2013). How best to use diagnostics to inform case or herd management is a key question for further research, whereby markers of inflammation, infection and AMR should be considered, as well as technological, biological and socio-economic aspects.

5.3 | Treatment

Recommendations for CM treatment have been reviewed relatively recently, considering antimicrobial treatment (Roberson, 2012) and non-steroidal anti-inflammatory drugs (Leslie & Petersson-Wolfe, 2012). Pathogen-specific reviews are available for *S. aureus* (Barkema, Schukken, & Zadoks, 2006), *E. coli* (Suojala et al., 2013) and *S. uberis* (Zadoks, 2007), but not for *Klebsiella* or *S. dysgalactiae*. The probability of cure for *S. dysgalactiae* mastitis was lower than for *S. uberis* mastitis in New Zealand (McDougall et al., 2007a, 2007b), whereas the opposite was true in the United States and Europe (Deluyker, Van Oye, & Boucher, 2005; Oliver et al., 2004), probably reflecting differences between dairy farming systems in herd management and mastitis epidemiology. Several studies suggest that *Klebsiella* mastitis does not respond to treatment as well as *E. coli* mastitis (Schukken et al., 2011a, 2012) and many veterinarians and farmers would confirm this from personal experience (Ostrum et al., 2008). In one study, the reported probability of cure was similar for *Klebsiella* and *E. coli* cases, but recurrence of CM and removal from the herd were more likely after *Klebsiella* mastitis (Oliveira et al., 2013). For mild-to-moderate coliform CM, there is fairly broad consensus that treatment has limited impact on the probability of cure (Hogan & Smith, 2003; Roberson, 2012; Suojala et al., 2013). The use of the 3/4GC ceftiofur and cefquinome, however, improved treatment outcomes in comparison with first-generation cephalosporins or no treatment, respectively (Schukken et al., 2011a, 2013). This adds complexity to the debate because it suggests that antimicrobial treatment of mild-to-moderate coliform mastitis may be beneficial, contradicting the prevailing paradigm. Others, however, did not observe a significant effect of ceftiofur treatment on clinical

or bacteriological cure of *E. coli* mastitis (Ganda et al., 2016a). Moreover, the use of 3/4GC in farming is strongly discouraged by WHO and several veterinary professional organizations (see Socio-economic and zoonotic aspects). In the authors' opinion, the arguments against use of 3/4GC for treatment of mild-to-moderate CM outweigh the arguments in favour.

Building on the desire to reduce AMU and the notion that antimicrobial treatment is likely to be beneficial for gram-positive mastitis but not for culture-negative mastitis or mastitis caused by gram-negative bacteria, *Mycoplasma*, *Prototheca* or yeast, the use of culture-based treatment decisions for mild-to-moderate CM has been advocated (Roberson, 2012; Suojala et al., 2013). Severe cases of CM, that is, those with systemic signs, should always be treated for the sake of cow welfare and to increase the likelihood of survival, and this may include systemic treatment (Suojala et al., 2013). Whether systemic antimicrobial treatment exerts its effect through clearance of IMI or through treatment of the bacteraemia that may accompany acute severe CM is not clear (Wenz et al., 2001). In the absence of systemic signs, treatment decisions for CM can be delayed for 24 hr without negative consequences for the animal. In that time, information on the causative pathogen can be generated. In North America, this is largely done through on-farm culture (Ganda et al., 2016b; Lago et al., 2011b). Elsewhere, this system that has not been adopted widely yet although methods for on-farm culture have been in evaluated in Europe (Mansion-de Vries et al., 2014; Viora et al., 2014) and Africa (Gitau et al., 2013). Alternative approaches include off-farm testing with 24-hr turnaround, which is rarely offered by mastitis diagnostic laboratories, and use of molecular methods, which are not available in on-farm format yet. On-farm culture methods use agar plates (Ganda et al., 2016b; Royster et al., 2014) or Petrifilms (McCarron et al., 2009) that include selective supplements to allow for culture of subsets of isolates only, for example, total bacterial, gram-negative, staphylococcal or GPCN growth. In the largest field study to evaluate the outcome of culture-based treatment, a significant reduction was observed in AMU (from 100% of CM cases treated with antimicrobials to 44%) without a significant impact on milk discard, clinical or bacteriological cure, new infections, SCC, milk yield or lactational survival (Lago et al., 2011a, 2011b). Thus, there were benefits in the form of reduced AMU and cost savings without demonstrable disadvantages of culture-based treatment. The argument could be made that the absence of negative effects in such field studies is due to lack of power rather than true absence of negative impacts and that on-farm culture should not be advocated. The counterargument would be that negative impacts would have been limited if they were not measurable and that the benefits of this approach outweigh the costs, particularly when restrictions on AMU are in place.

The incidence of environmental mastitis is particularly high around and during the dry period and parturition, when major changes occur in the cow's physiological, endocrinological and immunological status (Bradley et al., 2015b; Schukken et al., 2011b). To prevent new IMI during the dry period, farmers commonly use antimicrobial DCT, which was originally developed for long-term

treatment of existing IMI without the need to discard milk. DCT can be used for all cows, known as blanket DCT (bDCT), or for selected cows or quarters only (sDCT). Studies comparing bDCT versus sDCT were published as far back as the 1970s (Rindsig et al., 1978). Then, as in subsequent studies, sDCT was as effective at eliminating existing IMI as bDCT, but the risk of new infections was higher with sDCT (Rindsig et al., 1978; Schukken et al., 1993). For decades, the benefits of bDCT to cow health and welfare (i.e., the reduced risk of new infections) were thought to outweigh the risks in terms of AMR in many parts of Europe, with the exception of the Nordic countries where sDCT is the norm (Østeras, Edge, & Martin, 1999). In Denmark, DCT can only be administered after a case of CM within 30 days of dry-off or after detection of a pathogen in a milk sample, putting greater emphasis on clinical and microbiological criteria than on SCC (Bennedsgaard, Klaas, & Vaarst, 2010). Increasingly, other European countries, for example, the UK and the Netherlands, now no longer considered bDCT advisable or acceptable because of concerns over AMR (Biggs et al., 2016; Scherpenzeel et al., 2014). To select cows for DCT, a wide range of criteria have been considered, including SCC, CM and culture results for part or all of the current and/or previous lactations (Biggs et al., 2016; Cameron et al., 2014). One of the most simple criteria was used in a study of 97 herds in the Netherlands, where DCT was not used in cows that had low SCC at the last milk recording at dry-off (SCC <250,000 cells/ml for multiparous cows and <150,000 cells/ml for primiparous cows), without consideration of SCC or CM data from previous time points and without pathogen detection. Despite an increase in CM and associated antimicrobial treatment in animals that did not receive DCT, total antimicrobial use related to mastitis was reduced by 85% using this approach (Scherpenzeel et al., 2014). This demonstrates the feasibility of reduced AMU if we are willing to accept the impact on cow health and welfare.

To prevent new IMI in non-lactating animals, internal (Huxley et al., 2002) and external (Lim et al., 2007) teat-sealants and teat-dips (Lopez-Benavides et al., 2009) have been evaluated. Originally developed in the 1970s, internal sealants did not receive much attention in Europe until the 21st century (Huxley et al., 2002). They have subsequently been used in combination with antimicrobial DCT or as an alternative to antimicrobial DCT. Current teat-sealants do not contain compounds that treat existing IMI, but there is potential to combine them with immune-modifiers that speed up mammary gland involution or with a disinfectant such as chlorhexidine to reduce the risk of new infections (Compton, Emslie, & McDougall, 2014; Lanctôt et al., 2017). Whether such modifications provide any benefit over current internal teat-sealants remains to be demonstrated in field studies. Based on meta-analysis of 16 studies on internal teat-sealants, Rabiee and Lean (2013) reported a reduction in new dry-period IMI by 25% in studies with a positive control (antimicrobial treatment) and by 73% in studies with a negative control (no treatment), whilst CM was reduced by 29% and 48%, respectively. No effect on SCC or linear score was detected. The adoption of sDCT at national or herd level is influenced by the attitudes of farmers, veterinarians, the public and policymakers (Higgins et al.,

2017; Scherpenzeel et al., 2016). Knowledge alone is not enough, as many mastitis-related management practices that are generally considered to be important by experts are not widely used by farmers (Down et al., 2016). In recent years, evidence-based decision-making by veterinarians and communication of health-management advice have become topics of study in their own right (Higgins et al., 2016; Jansen & Lam, 2012). Involvement of farmer discussion groups may play an important role in empowering farmers and promoting udder health through hygiene measures when reducing use of antimicrobials in lactating and dry cows (Bennedsgaard et al., 2010). Whilst there is a need for development of better technical or biological tools for management of environmental mastitis, the importance of communication and incentives to support uptake of such tools must not be underestimated.

5.4 | Vaccination

Mastitis vaccine development has focussed primarily on *E. coli*, *S. uberis* and *S. aureus*. Criteria for evaluation of vaccination success include prevention or reduced severity of CM, reduced milk loss, reduced mortality and, for *S. aureus*, improved chances of cure and reduced transmission (Schukken et al., 2014; Smith, Lyman, & Anderson, 2006). Contagious transmission of *S. aureus* can largely be prevented through good herd management (Sommerhäuser et al., 2003), so vaccination is particularly relevant for prevention of environmental *S. aureus*. Attempts to develop *S. aureus* mastitis vaccines started in the 1960s, but products on the market today are still not satisfactory (Landin et al., 2015). In the foreseeable future, the dairy industry cannot rely on the magic bullet of vaccination for reduced AMU and improved mastitis control. Because of space constraints, we refer to recent reviews for further discussion of staphylococcal vaccines (Pereira et al., 2011) and general aspects of mastitis vaccine development (Bharathan & Mullarky, 2011; Erskine, 2012), whilst providing a brief discussion of mastitis vaccines for *E. coli* and GPCN cocci.

Vaccination against *E. coli* mastitis is commonly used in the United States (Erskine, 2012) and has recently been introduced in Europe. The effect of vaccination with a core J5 *E. coli* vaccine is probably largely based on antibodies, as reviewed recently, and cellular immunity may contribute (Schukken et al., 2011b). Effects of vaccination include reduced severity of mastitis and reduced yield losses, which is sufficient to offset the cost of vaccination and provides an estimated 2.56:1 return on investment (Bradley et al., 2015a; Schukken et al., 2011b). Although marketed as *E. coli* vaccine, the J5-vaccine may provide some protection from culling among cows with *Klebsiella* mastitis (Wilson et al., 2007). It is possible that this effect, as well as the observed reduction in severity of all coliform CM, is mediated through the systemic pathogenesis of severe coliform mastitis (Erskine, 2012). Vaccination does not reduce the negative impact of CM on reproduction, nor the overall number of CM cases (Wilson et al., 2007, 2008). The failure of current J5-vaccines to reduce incidence of *E. coli* IMI is their major limitation, and efforts are underway to enhance infection prevention through intramammary as opposed to systemic vaccination (Pomeroy

et al., 2016). Intramammary immunization may trigger mucosal immunity, and targeting mucosal immunity is seen as the next battle in development of mastitis vaccines (Bharathan & Mullarky, 2011). Meanwhile, use of J5-vaccines should be adapted to individual herd needs (Erskine, 2012).

Whereas production of opsonizing antibodies to promote neutrophil uptake and killing underpinned the success of current J5-vaccines against *E. coli* mastitis, it was recognized several decades ago that this approach is unlikely to be successful for *S. uberis* because increased antibody levels did not translate into increased opsonic activity (Hill et al., 1994). Alternative approaches to vaccine development have been explored since, including attempts to produce antibodies that would interfere with the metabolic needs of the bacteria and bacterial growth, for example, by binding plasminogen activator A (PauA) (Leigh, 1999). Others have focussed on production of antibodies that would interfere with binding of *S. uberis* to mammary epithelial cells, which is mediated by the *S. uberis* adhesion molecule (SUAM; Almeida et al., 2015; Prado et al., 2011). Antibodies induced through vaccination with recombinant SUAM inhibit adherence and internalization of *S. uberis* into mammary epithelial cells in vitro, but the importance of this mechanism is debated (Günther et al., 2016; Prado et al., 2011). Although *pauA* and *sua* genes are highly prevalent and highly conserved across strains of *S. uberis* (Perrig et al., 2015), mastitis can be caused by strains that are negative for *pauA* or contain frameshift mutations in *sua*, emphasizing the challenges posed by heterogeneity of the species (Gilchrist et al., 2013; Tassi et al., 2015). In addition to bacterial replication and adhesion, the role of mononuclear leucocytes has been a focus of *S. uberis* vaccine development. Vaccination with *S. uberis* enhances the proliferative response of peripheral blood lymphocytes to *S. uberis* antigens and induces an antigen-specific cytotoxic effect against blood monocytes/macrophages that have phagocytosed *S. uberis* (Hill et al., 1994; Wedlock et al., 2014). It is hoped that better understanding or manipulation of the cellular immune response to *S. uberis* may contribute to successful vaccine development (Denis et al., 2011; Schukken et al., 2011b), but it remains a challenge to activate and harness the cell-mediated arm of the immune response in the unique immunological environment of the mammary gland (Bharathan & Mullarky, 2011).

Attempts to develop vaccines against *S. agalactiae* mastitis were described in the early 1970s (Johnson & Norcross, 1971). Because of the successful control of contagious transmission of *S. agalactiae*, there has been little incentive for vaccine development in the Western world. Elsewhere, for example, in China, prevalence of *S. agalactiae* is still high, and *S. agalactiae* mastitis vaccine development is of renewed interest. Preliminary studies in mouse models show some promise (Liu et al., 2017), but the route from "fiction" (possibility) to "fact" (realization) is often a long one for mastitis vaccines (Yancey, 1999). Like research into pathophysiology and epidemiology, research into vaccine development for *S. dysgalactiae* is largely neglected. Encouraging preliminary reports on reduction of *S. dysgalactiae* infection in a dry-cow challenge model through use of the surface receptor protein GapC have not led to a vaccine, even

though there were hopes that such a vaccine could provide cross-protection to *S. dysgalactiae*, *S. agalactiae* and *S. uberis* (Bolton et al., 2004; Perez-Casal, Prysliak, & Potter, 2004). More recently, the polysaccharide envelope of *S. dysgalactiae* has been investigated as a potential vaccine target, starting with stereocontrolled synthesis of a tetrasaccharide repeating unit coupled to a T-cell-stimulating immunogen (Ghosh, Nishat, & Andreana, 2016). It will be interesting to see whether involvement of additional disciplines, such as chemistry, can bring the dream of gram-positive mastitis vaccines closer.

6 | CONCLUSION

In recent decades, there have been major changes in dairy farming and in the distribution of mastitis pathogens. Contagious transmission of mastitis can be controlled through good milking parlour hygiene, identification, treatment or culling of infected animals, and tools that reduce the probability of transmission after contact, such as teat disinfectants. The major impediment to successful implementation of those tools is the binary classification of bacterial species as contagious or environmental when in reality many bacterial species, notably *S. aureus* and *S. uberis*, can be transmitted in multiple ways. This insight has been derived from molecular studies, which allowed for strain typing of mastitis pathogens. Use of such methods as part of mastitis diagnostics could contribute to targeting of transmission prevention measures. Improved targeting is also needed for mastitis treatment to meet societal demands for the maintenance of good animal welfare with reduced use of antimicrobials, particularly highest priority antimicrobials such as third- and fourth-generation cephalosporins. Targeted or selective treatment of dry cows has been the norm in Nordic countries and is increasingly adopted elsewhere in Europe. There is a need for better tools and education on selection of cows for treatment, whereby both under- and overtreatment should be avoided. Selective treatment is also applied to clinical mastitis in lactation, where treatment decisions are guided by on-farm culture methods that have been developed and evaluated in the past decade. Improved methods for on-farm diagnostics with shorter time-to-result could promote uptake of such approaches beyond North America. Less progress has been made in vaccine development. Despite major research effort, currently available mastitis vaccines provide proven protection to damage resulting from coliform mastitis, but efficacy of gram-positive mastitis vaccines is lacking or debated at best. Vaccine development is hampered by the heterogeneity of mastitis-causing bacteria and by the unique immunological environment of the mammary gland. Existing tools to enhance host resistance to mastitis, such as breeding, nutrition and prevention of teat-end keratosis, continue to be important. A new area of science that has not been explored or exploited fully is the study of microbiota. Microbiota studies suggest that mastitis should possibly not be seen as intramammary infection of a sterile organ but as dysbiosis in the mammary gland. Manipulation of the microbiota of teats and mammary glands may provide new tools for prevention or correction of such dysbiosis. Reduction of exposure to environmental pathogens is a key component of environmental mastitis prevention. With changes in farm sizes and systems, mechanization, labour force and use of bedding materials, there is a need for better understanding of how pathogen accumulation can be prevented through management of the environment and the workforce. In doing so, not only bedding material but also the remainder of the indoor environment and the outdoor environment need to be considered. Last but possibly most importantly, technological or biological knowledge, tools and innovations need to be supported by appropriate communication and socio-economic incentives to enhance their uptake. Based on the above, three priority areas for further research are proposed:

1. Improved diagnostic tools for evidence-based targeting of antimicrobial treatment and transmission prevention measures;
2. Tools to monitor and manage bacterial exposure in the dairy cow environment and host resistance to such exposure, for example, through manipulation of the cow's microbiota.
3. Communication strategies and socio-economic incentives to influence knowledge and belief systems of veterinarians and farmers and to promote uptake of existing and new mastitis control tools.

Without use, no tool will support the sustainable intensification of dairy production that is needed to satisfy the growing demand from the world's human population.

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