An Update on Environmental Mastitis – Challenging Perceptions

Ilka C. Klaas[a], Ruth N. Zadoks[b]

[a] Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, DK-1870 Frederiksberg C, Denmark

[b] Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, EH26 0PZ, United Kingdom; and Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G61 1QH, United Kingdom

ABSTRACT

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 programs. 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 *E. 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.

Key words: environmental mastitis, molecular epidemiology, bedding, coliforms, streptococci, antimicrobial use

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 – a lot of 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, e.g. trough lower yields, increased culling, discarded milk, and impacts on fertility (Halasa et al., 2007; Seegers et al., 2003). In addition, mastitis affects animal welfare, which is highly valued in many industrialized countries (Byrd et al., 2017; Tremetsberger et al., 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 Organisation (WHO) recently endorsed a global action plan to tackle AMR and published a list of priority pathogens for research and development of new antibiotics (WHO 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, e.g. 3rd and 4th generation cephalosporins (3/4GC) and fluoroquinolones (" critical") (WHO, 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 paper 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.

DISEASE IN THE NATURAL HOST

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* are 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* (Zadoks et al., 2000; Tassi et al., 2013). 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 and 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 Epidemiology).

Gram-negative mastitis Mastitis caused by *E. coli* is generally transient and disease outcome largely depends on host factors, e.g. lactation stage (Burvenich et al., 2003), energy balance (Suriyasathaporn et al., 2000), vitamin deficiency (Smith et al., 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

111 isolates have demonstrated that *E. coli* infections can be persistent, often with repeated episodes of
112 CM linked by periods of subclinical infection (Döpfer et al., 1999). Subclinical coliform infections may
113 start in the dry period and can manifest as CM in early lactation, up to more than 100 days in milk
114 (Bradley and Green, 2000). In herds with bulk milk somatic cell count (BMSCC) below 250,000
115 cells/ml more than 50% of early lactation coliform CM were due to dry period infection (Bradley and
116 Green, 2000). The difference between onset of infection and clinical manifestation of disease has
117 been attributed to polarization of the immune response and anti-inflammatory signalling during the
118 dry period (Quesnell et al., 2012; Schukken et al., 2011b). Onset of infection in the dry period leading
119 to CM in lactation has also been observed for *Klebsiella, Citrobacter* and *Serratia* spp. (Bradley and
120 Green, 2000). For prevention of environmental mastitis, it is important to determine whether CM in
121 early lactation is due to infections during the dry period or during lactation. Control measures need
122 to target the relevant infection risks, e.g. poor environmental hygiene or non-use of teat sealants in
123 the dry period, versus inadequate hygiene or nutrition in lactation.
124 The pathophysiology of IMI due to *Klebsiella*, *Enterobacter* spp. and non-coliform
125 Enterobacteriaceae such as *Serratia* spp. is not n as well-studied as for *E. coli* but there is a recent
126 review dedicated to comparative analysis of their pathogenicity and immune response patterns
127 (Schukken et al., 2012). In experimental studies, *Klebsiella* elicits more severe clinical signs and a
128 stronger immune response than *E. coli*, whereby serum haptoglobin, interleukin (IL)-1 and IL-t
129 concentrations in serum are indicative of the chance of survival (Hisaeda et al., 2011). On-farm
130 mortality due to *Klebsiella* can be high (Ostrum et al., 2008; Schukken et al., 2012). Bacteraemia may
131 develop in cows with severe acute CM and contributes to mortality (Wenz et al., 2001; Suojala et al.,
132 2013). Bacteraemia may be caused by the mastitis-pathogen or by bacteria from the gut or lung, e.g.
133 *Pasteurella* or *Salmonella* spp. (Wenz et al., 2001). Subclinical and mild clinical manifestations of
134 *Klebsiella* mastitis also occur quite commonly (Oliveira et al., 2013; Figure 2). Knowledge of causative
135 agents of CM can inform management decisions, e.g. around vaccination or hygiene measures (see
136 Prevention, Detection and Control).

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 and colleagues (2015) noted that the type VI secretion system (T6SS) was present in all 4 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 and colleagues (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 (3 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 et al., 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

plasmid-borne 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.

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 garviae* (Cameron et al., 2016; Petersson-Wolfe et al., 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 and Gay, 1993; Smith et al., 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., 2013; Sampimon et al., 2009a).

Within *S. uberis*, multiple clonal complexes 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., 2015) and is associated with differences in uptake and killing by neutrophils or monocytes *in vitro* (Hill 1988, Tassi et al., 2013).

189 There is, however, no obvious association between outcome of infection and gene content (Hossain
190 et al., 2015). Strain-specific virulence has also been documented for *E. faecium* (Petersson-Wolfe et
191 al., 2009). Potential virulence genes underpinning such differences have not been studied in
192 Enterococci but the genomic tools that have been developed to study virulence factors of *S. uberis*
193 mastitis could provide insight into the functional genomics of other GPCN species (Blanchard et al.,
194 2015). As for coliforms, host-characteristics affect the outcome of GPCN infections: cows in early
195 lactation responded differently to *E. faecium* challenge than those in late lactation, and a *S. uberis*
196 strain that largely failed to cause infection in mid-lactation animals had been isolated from CM at
197 parturition (Petersson-Wolfe et al., 2009; Tassi et al., 2013). *In vitro*, macrophages from dry cow
198 secretion are more active against *S. uberis* than those from mid-lactation cows, even though *S.*
199 *uberis* infections commonly occur in the dry period (Denis et al., 2006). The role of mammary
200 epithelium in pathogenesis of *S. uberis* mastitis is debated and has various been described as linked
201 to infection outcome (Tassi et al., 2015), largely irrelevant (Leigh, 1999) or sufficiently common and
202 critical to base vaccine development around it (Almeida et al. 2015; see Vaccines).
203 *Streptococcus agalactiae* is currently not considered as one of the "environmental
204 streptococci" but we argue that this GPCN species may be of environmental origin with humans
205 acting as reservoir. Challenge studies comparing the bovine host response to *S. agalactiae* of human
206 and bovine origin were published in the early 1980s and are poorly known in the current mastitis
207 community (Jensen, 1982; Van den Heever and Giesecke, 1980). Inoculation of bovine mammary
208 glands with *S. agalactiae* from humans, where it is primarily known as Group B Streptococcus,
209 results in an acute response characterized by CM with milk losses greater than those observed after
210 challenge with bovine strains (Jensen, 1982; Van den Heever and Giesecke, 1980). Infections with
211 human strains are more likely to cure spontaneously than those caused by bovine strain (Jensen,
212 1982). This, combined with lower levels of bacterial shedding, limits the opportunity for contagious
213 transmission (Jensen, 1982). Strain specific shedding has also been documented in field studies
214 (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 USA, 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).

***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., 2011). The authors of a recent review (Reyher et al,. 2011) 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 physico-chemical 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 and Hillerton, 1995). In some modern large herds, e.g. in the High Plains area of the Western USA, 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 USA 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 cause or consequence of CM or antimicrobial

241 treatment is unknown (Falentin et al., 2016). Further research into the composition and role of teat
242 end microbiota, the impact of teat disinfectants and antimicrobial treatment, and potential
243 microbiota manipulations may provide new insight or tools for environmental mastitis control.
244
245 **EPIDEMIOLOGY**
246 ***Pathogen Characteristics*** Molecular epidemiology studies have been important in elucidating the
247 range of transmission modes within mastitis-causing pathogen species and it is increasingly clear
248 that the distinction between contagious and environmental pathogens should be applied at strain
249 level rather than species level (Gurjar et al., 2012; Zadoks et al., 2011a). *Streptococcus agalactiae*,
250 long considered the quintessential contagious pathogen, may originate from humans (Dogan et al.,
251 2005) or faeces (Farre et al., 2017; Jørgensen et al., 2016). *Klebsiella pneumoniae*, almost exclusively
252 seen as environmental pathogen, may occasionally spread from cow to cow (Munoz et al., 2007;
253 Schukken et al., 2011a). In human medicine, there is increasing recognition that most people carry *S.*
254 *aureus* and that patients may become infected with their own strain of the pathogen whilst staying
255 in the same hospital (Price et al., 2017). Likewise, cows staying on the same dairy farm may become
256 infected with their own individual or environmental strains of *S. aureus* (Zadoks et al., 2011a).
257 Control strategies that reduce contagious transmission do not affect the occurrence of
258 environmental *S. aureus* mastitis (Sommerhäuser et al., 2003). The possibility of contagious
259 transmission of *S. uberis* was demonstrated with molecular tools more than a decade ago, and it is
260 now acknowledged that cow-to-cow transmission may be the predominant route of infection in
261 many dairy herds (Davies et al., 2016; Zadoks et al., 2003). Veterinarians' and researchers' insistence
262 on erroneously classifying *S. aureus* as contagious pathogen and *S. uberis* as environmental
263 pathogen leads to false emphasis on mastitis control methods that may be irrelevant to a farm's
264 situation. For example, a major overhaul of the parlour routine will not resolve an environmental *S.*
265 *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 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 et al., 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.

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 to 40% of healthy men and women carrying the organism in their urogenital tract, gastro-intestinal 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

292 can be found in marine, fresh and waste water (reviewed in Delannoy et al., 2013). Within the species
293 *Streptococcus dysgalactiae,* two subspecies are recognized, i.e. *S. dysgalactiae* subsp. *equisimilis* and
294 *S. dysgalactiae* subsp. *dysgalactiae*. The former is a commensal and pathogen of people but rarely
295 affects cattle. The latter is a frequent mastitis pathogen, and commonly referred to as *S. dysgalactiae*
296 in the veterinary literature (Lundberg et al., 2014). In sheep, *S. dysgalactiae* subsp. *dysgalactiae* causes
297 polyarthritis or joint ill in lambs, but it rarely causes mastitis. *S. uberis* and *E. coli* are also common
298 mastitis pathogens in cattle but relatively rare in sheep (Gelasakis et al., 2015; Zadoks et al., 2014).
299 Conversely, *Mannheimia haemolytica* mastitis is common in sheep but not in cattle, whereas *S. aureus*
300 is common in both host species (Gelasakis et al., 2015; Zadoks et al., 2011a) and also in people (Price
301 et al, 2017; Zadoks et al., 2014). The mechanisms underpinning observed differences in host
302 preference are poorly studied and may provide insights into host-adaptation or virulence factors. Pigs,
303 dogs and cats may occasionally act as sources of mastitis pathogens, with pigs playing a role in MRSA
304 transmission (see Socio-economic aspects), and dogs or cats acting as a source of *S. canis* (Richards et
305 al., 2012).
306
307 ***Vectors*** Mastitis pathogens are rarely vector-transmitted. Insect vectors such as flies and wasps may
308 play a role in transmission of some mastitis pathogens, notably *S. aureus, S. dysgalactiae* and
309 pathogens associated with summer mastitis (Chirico et al., 1997; Yeruham et al., 2002). Vector-borne
310 mastitis may affect non-lactating cattle but should probably be classed as contagious mastitis
311 because pathogens are transmitted from host to host by the vectors (Owens et al., 1998). A role of
312 stable flies in transmission of *E. coli* mastitis has been suggested but not proven (Castro et al., 2016).
313 Environmental controls, i.e. insect control, may reduce the risk of vector-borne mastitis but an
314 investigation of the impact of fly control in heifers on early lactation CM yielded results that
315 depended on selection of the outcome variable of interest (Green et al., 2007).
316

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 et al., 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 or raw manure (Husfeldt et al., 2012). Control methods may differ between categories of pathogens, both for RMS (Leach et al., 2015; Rowbotham and Ruegg, 2016) or CBP (Eckelkamp et al., 2016). Leach and colleagues (2015) warn that caution is needed when adopting RMS use in Europe, i.e. under climatic conditions that differ from the dry climates in the USA 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, e.g. sand, is recommended to reduce the environmental load of opportunistic pathogens but high loads of *E. coli*,

343 *Klebsiella* and GPCN cocci have been found in sand bedding (Kristula et al., 2005; Munoz et al.,
344 2006). High bacterial counts can result from on-farm recycling of sand or poor bedding management.
345 Once mixed with manure, any type of bedding becomes a source of pathogens and the use of sand
346 may give a false sense of security, leading to poor maintenance of stalls (Figure 4). Barn conditions
347 rather than bedding type may be the main determinants of bacterial counts (Zehner et al., 1986).
348 Faecal shedding has been documented for *S. agalactiae* (Jørgensen et al., 2016), *S. uberis*
349 (Zadoks et al., 2005) and *Klebsiella* (Munoz e al., 2006) and average faecal prevalence ranges from
350 5% to 23% and >80%, respectively, with considerable differences between farms. The faecal
351 prevalence of *S. aureus* in cattle ranges from 1.4 to 12%, based on testing of faecal swabs
352 (Dimitracopoulos et al., 1977; Roberson et al., 1994). Faecal contamination turns not just bedding
353 but also alleys, traffic lanes, water troughs and the outdoor environment into sources of
354 environmental pathogens. *S. agalactiae* has been found in milking parlours, alleys, stalls and water
355 troughs (Jørgensen et al., 2016). *S. uberis* can be found in bedding, traffic lanes, water troughs, and
356 the outdoor environment, including soil and grass (Lopez-Benavides et al., 2007; Zadoks et al., 2005).
357 In the absence of cattle, *S. uberis* is undetectable, or levels decline rapidly, implying that cattle, and
358 most likely cattle faeces, are the original source of environmental *S. uberis* (Lopez-Benavides et al.,
359 2007; Zadoks et al,. 2005). The prevalence of *Klebsiella* in beds, alleys, on legs and on teats is very
360 similar to the level of faecal shedding in the same herd, whilst a higher prevalence was detected in
361 drinking water and a lower prevalence in feed (Zadoks et al., 2011b).The presence of gram-negative
362 and gram-positive organisms in faeces and drinking water suggests that an oro-faecal transmission
363 cycle exists for several major mastitis pathogens, including *S. agalactiae* and *K. pneumoniae*
364 (Jørgensen et al., 2016; Zadoks et al., 2011b). With the exception of *S. agalactiae*, there was
365 considerable strain heterogeneity within environmental sources of pathogens, which can be
366 attributed to between and within-animal heterogeneity of strains in faeces (Munoz and Zadoks,
367 2006; Zadoks et al., 2005). The faecal bacterial load in the environment is a function of initial

368 contamination, subsequent amplification and removal and can be managed to reduce the challenge
369 to the cows' immune system (see Prevention, Detection and Control).
370
371 **SOCIO-ECONOMIC IMPACT AND ZOONOTIC ASPECTS**
372 In addition to societal pressures outlined in the Introduction, there are financial pressures on dairy
373 farming. When supermarkets charge more for soft drinks, which are essentially bottles of water with
374 additives, than for a bottle of milk produced by sentient beings, the financial pressures on dairy
375 production become visible: Not only do we need to produce "more with less" in terms of physical
376 resources, we also need to produce "more with less" in terms of financial and human resources
377 (Figure 5). Non-antimicrobial control of bacterial IMI is both labour intensive and knowledge
378 intensive and the shortage of appropriately trained staff is an increasing problem (Maloney, 2002;
379 Tipples and Trafford, 2011). In some countries, expensive labour is replaced by automation, e.g. of
380 milking machines or alley scrapers, whilst dairy care and milk harvesting rely heavily on indigenous
381 or foreign human labour in other countries (Barkema et al., 2015).
382 Direct and indirect economic losses due to mastitis have been estimated (Halasa et al., 2007)
383 and vary greatly between animals and pathogens. For example, yield losses in heifers are greatest
384 after *E. coli* CM and in multiparous animals after *Klebsiella* CM, and both coliforms have greater
385 impact on fertility than other pathogen species (Hertl et al., 2014a,b). Yield losses may persist for
386 months after coliform or GPCN mastitis whilst CM with non-aureus Staphylococci does not cause
387 reduced production (Hertl et al., 2014a). The association of pathogens with culling risk differs
388 between heifers and multiparous animals, lactation stages, and number of CM episodes, with
389 different combinations of factors identifying different pathogen species as being associated with the
390 highest risk of culling (Cha et al., 2013; Gröhn et al., 2005). Other costs of mastitis are even harder to
391 quantify and relate to its impact on public perception, notably perceptions around animal welfare
392 and use of antimicrobials. This creates a dilemma as treatment of mastitis may be necessary for
393 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 betalactamase (ESBL) producing coliforms. Studies based on data collected around the millennium (1994 to 2001) showed little evidence for a relationship between use of antimicrobials for mastitis control and AMR (Erskine et al., 2002; Makovec and 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 and 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 and 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 1427 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-producers (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., 2013). 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 USA, 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.

PREVENTION, DETECTION AND CONTROL

Biosecurity External biosecurity, i.e. 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 health care products may be a source of pathogens, as described before for *Klebsiella* in sawdust and *Pasteurella* in teat wipes. Presence of pathogens in a health care product doesn't 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, i.e. 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 USA. Regardless of whether a wet (pre-dip used) or

446 dry (no pre-dip used) pre-milking routine is adopted, it is important to evaluate the effect of the
447 procedure. Scoring tools for cow, udder and teat cleanliness have been developed to assist with this
448 task and their use has demonstrated an association between dirty udders and risk of new infection
449 (Dohmen et al., 2010) or high bacterial counts on teats (Guarin et al., 2017; Munoz et al., 2008).
450 Moreover, it has been shown that the efficacy of pre-milking teat disinfection is lower for dirty teats
451 than for clean teats (Munoz et al,. 2008; Zdanowicz et al., 2004). Scoring systems have also been
452 developed for teat-end callosity or hyperkeratosis (Shearn and Hillerton, 1996). In a small study (135
453 cows), teat end hyperkeratosis was not associated with the risk of mastitis but a large study (1,667
454 cows) showed that severe hyperkeratosis is associated with increased risk of *E. coli* or *S. uberis* CM,
455 and moderate hyperkeratosis with increased risk of *E. coli* CM (Breen et al., 2009; Zoche-Golob et al.,
456 2015). Bacterial loads of both organisms are higher in teat ends with hyperkeratosis than in those
457 without, providing a plausible biological mechanism for the observed association (Paduch et al.,
458 2012). Hyperkeratosis is associated with the duration of milking, and particularly with overmilking,
459 which may be an issue in large parlours if automated cluster removal is not used or settings are
460 incorrect (Edwards et al., 2013). Thus, although milking machine settings and parlour routines are
461 primarily associated with contagious mastitis, they do also impact on the risk of environmental
462 mastitis. With the introduction of automated milking systems (AMS), the milking frequency is
463 increased, particularly for high yielding cows. This may be beneficial, through frequent removal of
464 bacteria and replenishment of somatic cells in the mammary gland, or harmful due to frequent
465 opening of the teat canal. Milk leakage is more common in cows milked by AMS than in a milking
466 parlour, particularly for cows with high milk flow (Klaas et al., 2005; Persson-Waller et al., 2003). This
467 could lead to higher risk for the cow itself, or for other cows in the herd if the cow leaks milk with
468 high bacterial loads (Munoz et al., 2007). Although consensus on udder health benefits may not
469 exist, AMS are gaining ground.

470 Tools to manage bacterial counts in bedding include bedding replacement and the use of
471 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 et al., 2013; Proietto et al., 2013). When using lime, both positive and negative effects were observed at teat level, i.e. 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 et al., 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 et al., 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 and Ruegg et al., 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 and colleagues (2007) grouped risk factors, which include protective factors, by stage of the dry period, i.e. 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,

498 the association between dry period IMI and lactational CM (see Disease in the Natural Host) suggests
499 that prevention of dry period IMI through reduced exposure to pathogens explains the observations,
500 at least in part. For cows that were out on pasture during the dry period, a pasture rotation method
501 of 2 weeks of grazing by dry cows followed by 4 weeks without grazing reduced the risk of early
502 lactation CM (Green et al., 2007). This may be due to a reduction in bacterial load on pasture in the
503 absence of cattle, as demonstrated for *S. uberis* (see Reservoirs). Specific advice on straw yard
504 management, which was used to house more than half of the cattle in the study, could not be
505 derived from the data (Green et al., 2007).
506
507 ***Detection*** Diagnostics can be used to detect mastitis, i.e. mammary gland inflammation, or IMI, i.e.
508 pathogen presence. Inflammation can be detected based on somatic cell count (SCC), electrolytes,
509 enzymatic markers or acute phase proteins (Viguier et al., 2009; Pyörälä et al., 2011). As methods for
510 pathogen detection become more sensitive, the ability to differentiate between pathogen-positive
511 samples with and without evidence of inflammation becomes increasingly important, particularly
512 when testing is conducted to inform treatment decisions, bearing in mind the societal pressure to
513 reduce AMU. Detection of IMI has traditionally been based on culture but there is a wide range of
514 opinions on how to interpret culture results (Dohoo et al., 2011). Species identity of cultured
515 bacteria can be confirmed with phenotypic or genotypic methods (Zadoks and Watts, 2009).
516 Phenotypic identification using biochemical profiles is unreliable for many mastitis pathogens,
517 including *Klebsiella* and *Staphylococcus* spp. (Munoz et al., 2007; Sampimon et al., 2009b). Modern
518 phenotypic testing is increasingly based on proteomics, notably matrix-assisted laser desorption
519 ionization time-of-flight mass spectrometry analysis (Cameron et al., 2017; Schabauer et al., 2014).
520 Its application directly to milk samples may be possible but only at high bacterial concentrations
521 (Barreiro et al., 2017). PCR or sequencing of housekeeping genes for species detection or
522 identification are commonly applied to milk samples (PCR) and cultured isolates (sequencing),
523 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, e.g. positive PCR-results due to sample contamination (Koskinen et al., 2010). Moreover, work on milk microbiota has shown that several mastitis-causing organims 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 has 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 turn-around-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-

550 negative mastitis or mild to moderate gram-negative mastitis (see Treatment). Possibly of greater
551 importance for test-uptake is farmer perception of what constitutes a useful test. In the
552 Netherlands, 34% of farmers submit milk samples from CM to a diagnostic laboratory, whereas 71%
553 would consider use of an on-farm test, depending on the time-to-result (Griffioen et al., 2016). A
554 relatively novel mastitis-diagnostic with potential for short time-to-result is loop-mediated
555 isothermal amplification (LAMP). LAMP-primers are available for *S. aureus* (Sheet et al., 2016) and
556 major streptococci (Bosward et al., 2016; Wang and Liu, 2015) and implementation as pregnancy-
557 test-like lateral flow device is possible (Cornelissen et al., 2016). A major challenge for on-farm
558 molecular detection of pathogens or AMR-genes is the large number of bacterial species and
559 resistance genes that may be present in milk or mastitis pathogens. There is a single penicillin-
560 resistance gene in *S. aureus*, which is covered by commercially available PCR, but there are multiple
561 categories of ESBL-genes in coliforms (bla_{SHV}, bla_{TEM} and bla_{CTX-M} ESBL), with multiple clusters of
562 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
563 al., 2013). How best to use diagnostics to inform case or herd management is a key question for
564 further research, whereby markers of inflammation, infection and AMR should be considered, as
565 well as technological, biological and socio-economic aspects.
566
567 ***Treatment*** Recommendations for CM treatment have been reviewed relatively recently, considering
568 antimicrobial treatment (Roberson, 2012) and non-steroidal anti-inflammatory drugs (Leslie and
569 Petersson-Wolfe, 2012). Pathogen-specific reviews are available for *S. aureus* (Barkema et al., 2006),
570 *E. coli* (Suojala et al., 2013) and *S. uberis* (Zadoks, 2007), but not for *Klebsiella* or *S. dysgalactiae*. The
571 probability of cure for *S. dysgalactiae* mastitis was lower than for *S. uberis* mastitis in New Zealand
572 (McDougall et al., 2007a,b) whereas the opposite was true in the USA and Europe (Deluyker et al.,
573 2005; Oliver et al., 2004), probably reflecting differences between dairy farming systems in herd
574 management and mastitis epidemiology. Several studies suggest that *Klebsiella* mastitis does not
575 respond to treatment as well as *E. coli* mastitis (Schukken et al., 2011a, 2012) and many

576 veterinarians and farmers would confirm this from personal experience (Ostrum et al., 2008). In one
577 study, the reported probability of cure was similar for *Klebsiella* and *E. coli* cases but recurrence of
578 CM and removal from the herd were more likely after *Klebsiella* mastitis (Oliveira et al., 2013). For
579 mild to moderate coliform CM, there is fairly broad consensus that treatment has limited impact on
580 the probability of cure (Hogan and Smith, 2003; Roberson, 2012; Suojala et al., 2013). The use of the
581 3/4GC ceftiofur and cefquinome, however, improved treatment outcomes in comparison to 1st
582 generation cephalosporins or no treatment, respectively (Schukken et al., 2011a, 2013). This adds
583 complexity to the debate because it suggests that antimicrobial treatment of mild to moderate
584 coliform mastitis may be beneficial, contradicting the prevailing paradigm. Others, however, did not
585 observe a significant effect of ceftiofur treatment on clinical or bacteriological cure of *E. coli* mastitis
586 (Ganda et al., 2016a). Moreover, the use of 3/4GC in farming, is strongly discouraged by WHO and
587 several veterinary professional organizations (see Socio-economic and zoonotic aspects). In the
588 authors' opinion, the arguments against use of 3/4GC for treatment of mild to moderate CM
589 outweigh the arguments in favour.

590 Building on the desire to reduce AMU and the notion that antimicrobial treatment is likely to
591 be beneficial for gram-positive mastitis but not for culture-negative mastitis or mastitis caused by
592 gram-negative bacteria, *Mycoplasma*, *Prototheca* or yeast, the use of culture-based treatment
593 decisions for mild to moderate CM has been advocated (Roberson 2012; Suojala et al., 2013). Severe
594 cases of CM, i.e. those with systemic signs, should always be treated for the sake of cow welfare and
595 to increase the likelihood of survival, and this may include systemic treatment (Suojala et al., 2013).
596 Whether systemic antimicrobial treatment exerts its effect through clearance of IMI or through
597 treatment of the bacteraemia that may accompany acute severe CM is not clear (Wenz et al., 2001).
598 In the absence of systemic signs, treatment decisions for CM can be delayed for 24 hours without
599 negative consequences for the animal. In that time, information on the causative pathogen can be
600 generated. In North America, this is largely done through on-farm culture (Lago et al., 2011b; Ganda
601 et al., 2016b). Elsewhere, this system that has not been adopted widely yet although methods for

602 on-farm culture have been in evaluated in Europe (Mansion-de Vries et al., 2014; Viora et al., 2014)
603 and Africa (Gitau et al., 2013). Alternative approaches include off-farm testing with 24-hr turn-
604 around, which is rarely offered by mastitis diagnostic laboratories, and use of molecular methods,
605 which are not available in on-farm format yet. On-farm culture methods use agar plates (Ganda et
606 al., 2016b; Royster et al., 2014) or Petri-films (McCarron et al., 2009) that include selective
607 supplements to allow for culture of subsets of isolates only, e.g. total bacterial, gram-negative ,
608 staphylococcal or GPCN growth. In the largest field study to evaluate the outcome of culture-based
609 treatment, a significant reduction was observed in AMU (from 100% of CM cases treated with
610 antimicrobials to 44%) without a significant impact on milk discard, clinical or bacteriological cure,
611 new infections, SCC, milk yield or lactational survival (Lago et al., 2011a, b). Thus, there were
612 benefits in the form of reduced AMU and cost-savings without demonstrable disadvantages of
613 culture-based treatment. The argument could be made that the absence of negative effects in such
614 field studies is due to lack of power rather than true absence of negative impacts and that on-farm
615 culture should not be advocated. The counterargument would be that negative impacts would have
616 been limited if they were not measurable and that the benefits of this approach outweigh the costs,
617 particularly when restrictions on AMU are in place.
618 The incidence of environmental mastitis is particularly high around and during the dry period
619 and parturition, when major changes occur in the cow's physiological, endocrinological and
620 immunological status (Bradley et al. 2015b; Schukken et al., 2011b). To prevent new IMI during the
621 dry period, farmers commonly use antimicrobial DCT, which was originally developed for long-term
622 treatment of existing IMI without the need to discard milk. DCT can be used for all cows, known as
623 blanket DCT (bDCT), or for selected cows or quarters only (sDCT). Studies comparing bDCT versus
624 sDCT were published as far back as the 1970s (Rindsig et al., 1978). Then, as in subsequent studies,
625 sDCT was as effective at eliminating existing IMI as bDCT, but the risk of new infections was higher
626 with sDCT (Rindsig et al., 1978; Schukken et al., 1993). For decades, the benefits of bDCT to cow
627 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 (Østerås et al., 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 et al., 2010). Increasingly, other European countries, e.g. 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 has 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 antibiotic 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 et al., 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)

654 and by 73% in studies with a negative control (no treatment), while CM was reduced by 29% and
655 48%, respectively. No effect on SCC or linear score was detected. The adoption of sDCT at national
656 or herd-level is influenced by the attitudes of farmers, veterinarians, the public and policy makers
657 (Higgins et al., 2017; Scherpenzeel et al., 2016). Knowledge alone is not enough, as many mastitis-
658 related management practices that are generally considered to be important by experts are not
659 widely used by farmers (Down et al., 2016). In recent years, evidence-based decision-making by
660 veterinarians and communication of health-management advice have become topics of study in
661 their own right (Higgins et al., 2016; Jansen and Lam, 2012). Involvement of farmer discussion
662 groups may play an important role in empowering farmers and promoting udder health through
663 hygiene measures when reducing use of antimicrobials in lactating and dry cows (Bennedsgaard et
664 al., 2010). Whilst there is a need for development of better technical or biological tools for
665 management of environmental mastitis, the importance of communication and incentives to
666 support uptake of such tools must not be underestimated.
667
668 ***Vaccination*** Mastitis vaccine-development has focussed primarily on *E. coli*, *S. uberis* and *S. aureus*.
669 Criteria for evaluation of vaccination success include prevention or reduced severity of CM, reduced
670 milk loss, reduced mortality and, for *S. aureus*, improved chances of cure and reduced transmission
671 (Schukken et al., 2014; Smith et al., 2006). Contagious transmission of *S. aureus* can largely be
672 prevented through good herd management (Sommerhäuser et al., 2003) so vaccination is
673 particularly relevant for prevention of environmental *S. aureus*. Attempts to develop *S. aureus*
674 mastitis vaccines started in the 1960s but products on the market today are still not satisfactory
675 (Landin et al., 2015). In the foreseeable future, the dairy industry cannot rely on the magic bullet of
676 vaccination for reduced AMU and improved mastitis control. Because of space constraints, we refer
677 to recent reviews for further discussion of staphylococcal vaccines (Pereira et al., 2011) and general
678 aspects of mastitis vaccine development (Barathan and Mullarky, 2011; Erskine, 2012), whilst
679 providing a brief discussion of mastitis vaccines for *E. coli* and GPCN cocci.

680 Vaccination against *E. coli* mastitis is commonly used in the USA (Erskine, 2012) and has
681 recently been introduced in Europe. The effect of vaccination with a core J5 *E. coli* vaccine is probably
682 largely based on antibodies, as reviewed recently, and cellular immunity may contribute (Schukken et
683 al., 2011b). Effects of vaccination include reduced severity of mastitis and reduced yield losses, which
684 is sufficient to offset the cost of vaccination and provides an estimated 2.56:1 return on investment
685 (Bradley et al., 2015a; Schukken et al., 2011b). Although marketed as *E. coli* vaccine, the J5-vaccine
686 may provide some protection from culling among cows with *Klebsiella* mastitis (Wilson et al., 2007). It
687 is possible that this effect, as well as the observed reduction in severity of all coliform CM, is mediated
688 through the systemic pathogenesis of severe coliform mastitis (Erskine, 2012). Vaccination does not
689 reduce the negative impact of CM on reproduction, nor the overall number of CM cases (Wilson et al.,
690 2007, 2008). The failure of current J5-vaccines to reduce incidence of *E. coli* IMI is their major limitation
691 and efforts are underway to enhance infection prevention through intramammary as opposed to
692 systemic vaccination (Pomeroy et al., 2016). Intramammary immunization may trigger mucosal
693 immunity, and targeting mucosal immunity is seen as the next battle in development of mastitis
694 vaccines (Bharathan and Mullarky, 2011). Meanwhile, use of J5-vaccines should be adapted to
695 individual herd needs (Erskine, 2012).
696 Whereas production of opsonising antibodies to promote neutrophil uptake and killing
697 underpinned the success of current J-5 vaccines against *E. coli* mastitis, it was recognized several
698 decades ago that this approach is unlikely to be successful for *S. uberis* because increased antibody
699 levels did not translate into increased opsonic activity (Hill et al., 1994). Alternative approaches to
700 vaccine development have been explored since, including attempts to produce antibodies that
701 would interfere with the metabolic needs of the bacteria and bacterial growth, e.g. by binding
702 plasminogen activator A (PauA) (Leigh, 1999). Others have focussed on production of antibodies
703 that would interfere with binding of *S. uberis* to mammary epithelial cells, which is mediated by the
704 *S. uberis* adhesion molecule (SUAM; Almeida et al., 2015; Prado et al., 2011). Antibodies induced
705 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 (Prado et al., 2011, Günther et al., 2016). 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 frame-shift 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 and Mullarky, 2011).

Attempts to develop vaccines against *S. agalactiae* mastitis were described in the early 70s (Johnson and 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, e.g. 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 et al., 2004). More recently, the polysaccharide envelop 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 et al., 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.

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 3rd and 4th 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 cultures 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

758 is lacking or debated at best. Vaccine development is hampered by the heterogeneity of mastitis-
759 causing bacteria and by the unique immunological environment of the mammary gland. Existing
760 tools to enhance host resistance to mastitis, such as breeding, nutrition and prevention of teat end
761 keratosis, continue to be important. A new area of science that has not been explored or exploited
762 fully is the study of microbiota. Microbiota studies suggest that mastitis should possibly not be seen
763 as intramammary infection of a sterile organ but as dysbiosis in the mammary gland. Manipulation
764 of the microbiota of teats and mammary glands may provide new tools for prevention or correction
765 of such dysbiosis. Reduction of exposure to environmental pathogens is a key component of
766 environmental mastitis prevention. With changes in farm sizes and systems, mechanisation, labour
767 force and use bedding materials, there is a need for better understanding of how pathogen
768 accumulation can be prevented through management of the environment and the work force. In
769 doing so, not only bedding material but also the remainder of the indoor environment and the
770 outdoor environment need to be considered. Last but possibly most importantly, technological or
771 biological knowledge, tools and innovations need to be supported by appropriate communication
772 and socio-economic incentives to enhance their uptake. Based on the above, three priority areas for
773 further research are proposed:
774
775 1. Improved diagnostic tools for evidence-based targeting of antimicrobial treatment and
776 transmission prevention measures;
777 2. Tools to monitor and manage bacterial exposure in the dairy cow environment and host
778 resistance to such exposure, e.g. through manipulation of the cow's microbiota.
779 3. Communication strategies and socio-economic incentives to influence knowledge and belief
780 systems of veterinarians and farmers and to promote uptake of existing and new mastitis control
781 tools.
782

783 Without use, no tool will support the sustainable intensification of dairy production that is needed to
784 satisfy the growing demand from the world's human population.
785
786 **ACKNOWLEDGEMENTS**
787 This paper was developed under the DISCONTOOLS framework (www.discontools.eu).
788
789 **REFERENCES**
790
791 1. Addis MF, Tanca A, Uzzau S, *et al.* The bovine milk microbiota: insights and perspectives from -
792 omics studies. Mol Biosyst. 2016;12(8):2359-72.
793 2. Ali T, Ur Rahman S, Zhang L, *et al.* ESBL-producing *Escherichia coli* from cows suffering mastitis
794 in China contain clinical class 1 integrons with CTX-M linked to ISCR1. Front Microbiol.
795 2016;7:1931.
796 3. Almeida RA, Kerro-Dego O, Prado ME, *et al.* Protective effect of anti-SUAM antibodies on
797 *Streptococcus uberis* mastitis. Vet Res. 2015;46:133.
798 4. Barkema HW, Schukken YH, Lam TJ, *et al.* Incidence of clinical mastitis in dairy herds grouped
799 in three categories by bulk milk somatic cell counts. J Dairy Sci. 1998;81(2):411-9.
800 5. Barkema HW, Schukken YH, Zadoks RN. Invited Review: The role of cow, pathogen, and
801 treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. J
802 Dairy Sci. 2006;89(6):1877-95.
803 6. Barkema HW, von Keyserlingk MA, Kastelic JP, *et al.* Invited review: Changes in the dairy
804 industry affecting dairy cattle health and welfare. J Dairy Sci. 2015;98(11):7426-45.
805 7. Barreiro JR, Gonçalves JL, Braga PA, *et al.* Non-culture-based identification of mastitis-causing
806 bacteria by MALDI-TOF mass spectrometry. J Dairy Sci. 2017;100(4):2928-2934.
807 8. Bennedsgaard TW, Klaas IC, Vaarst M. Reducing use of antimicrobials – Experiences from an
808 intervention study in organic dairy herds in Denmark. Livestock Sci. 2010;131:183-192.

9. Bharathan M, Mullarky IK. Targeting mucosal immunity in the battle to develop a mastitis vaccine. J Mammary Gland Biol Neoplasia. 2011;16(4):409-19.

10. Biggs A, Barrett D, Bradley A, *et al.* Antibiotic dry cow therapy: where next? Vet Rec. 2016;178(4):93-4.

11. Black RA, Taraba JL, Day GB, *et al.* The relationship between compost bedded pack performance, management, and bacterial counts. J Dairy Sci. 2014;97(5):2669-79.

12. Blanchard AM, Egan SA, Emes RD, *et al.* PIMMS (Pragmatic Insertional Mutation Mapping System) laboratory methodology a readily accessible tool for identification of essential genes in *Streptococcus*. Front Microbiol. 2016;7:1645.

13. Blum S, Heller ED, Krifucks O, *et al.* Identification of a bovine mastitis *Escherichia coli* subset. Vet Microbiol. 2008;132(1-2):135-48.

14. Bolton A, Song XM, Willson P, *et al.* Use of the surface proteins GapC and Mig of *Streptococcus dysgalactiae* as potential protective antigens against bovine mastitis. Can J Microbiol. 2004;50(6):423-32.

15. Bosward KL, House JK, Deveridge A, *et al.* Development of a loop-mediated isothermal amplification assay for the detection of *Streptococcus agalactiae* in bovine milk. J Dairy Sci. 2016;99(3):2142-50.

16. Bradley AJ, Green MJ. A study of the incidence and significance of intramammary enterobacterial infections acquired during the dry period. J Dairy Sci. 2000;83(9):1957-65.

17. Bradley AJ, Breen JE, Payne B, *et al.* An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom. J Dairy Sci. 2015a;98(3):1706-20.

18. Bradley AJ, De Vliegher S, Green MJ, *et al.* An investigation of the dynamics of intramammary infections acquired during the dry period on European dairy farms. J Dairy Sci. 2015b;98(9):6029-47.

19. Breen JE, Green MJ, Bradley AJ. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. J Dairy Sci. 2009;92(6):2551-61.
20. Burvenich C, Van Merris V, Mehrzad J, *et al.* Severity of *E. coli* mastitis is mainly determined by cow factors. Vet Res. 2003;34(5):521-64.
21. Byrd E, Widmar NO, Fulton J. Of fur, feather, and fin: Human's use and concern for non-human species. Animals. 2017;7(3):pii:E22.
22. Cameron M, McKenna SL, MacDonald KA, *et al.* Evaluation of selective dry cow treatment following on-farm culture: risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. J Dairy Sci. 2014;97(1):270-84.
23. Cameron M, Saab M, Heider L, *et al.* Antimicrobial susceptibility patterns of environmental streptococci recovered from bovine milk samples in the Maritime provinces of Canada. Front Vet Sci. 2016;3:79.
24. Cameron M, Barkema HW, De Buck J, *et al.* Identification of bovine-associated coagulase-negative staphylococci by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a direct transfer protocol. J Dairy Sci. 2017;100(3):2137-2147.
25. Capuco AV, Bright SA, Pankey JW, *et al.* Increased susceptibility to intramammary infection following removal of teat canal keratin. J Dairy Sci. 1992;75(8):2126-30.
26. Castro BG, Souza MMS, Regua-Mangia AH, *et al.* Genetic relationship between *Escherichia coli* strains isolated from dairy mastitis and from the stable fly *Stomoxys calcitrans*. Pesq. Vet. Bras. 2016;36(6):479-484.
27. Cha E, Hertl JA, Schukken YH, *et al.* The effect of repeated episodes of bacteria-specific clinical mastitis on mortality and culling in Holstein dairy cows. J Dairy Sci. 2013;96(8):4993-5007.
28. Chirico J, Jonsson P, Kjellberg S, *et al.* Summer mastitis experimentally induced by *Hydrotaea irritans* exposed to bacteria. Med Vet Entomol. 1997;11(2):187-92.

29. Compton CW, Emslie FR, McDougall S. Randomised controlled trials demonstrate efficacy of a novel internal teat sealant to prevent new intramammary infections in dairy cows and heifers. N Z Vet J. 2014;62(5):258-66.
30. Cornelissen JB, De Greeff A, Heuvelink AE, *et al.* Rapid detection of *Streptococcus uberis* in raw milk by loop-mediated isothermal amplification. J Dairy Sci. 2016;99(6):4270-81.
31. Cortinhas CS, Tomazi T, Zoni MS, *et al.* Randomized clinical trial comparing ceftiofur hydrochloride with a positive control protocol for intramammary treatment of nonsevere clinical mastitis in dairy cows. J Dairy Sci. 2016;99(7):5619-28.
32. Dahmen S, Métayer V, Gay E, *et al.* Characterization of extended-spectrum beta-lactamase (ESBL)-carrying plasmids and clones of Enterobacteriaceae causing cattle mastitis in France. Vet Microbiol. 2013;162(2-4):793-9.
33. Daly M, Power E, Björkroth J, *et al.* Molecular analysis of *Pseudomonas aeruginosa*: epidemiological investigation of mastitis outbreaks in Irish dairy herds. Appl Environ Microbiol. 1999;65(6):2723-9.
34. Davies PL, Leigh JA, Bradley AJ, *et al.* Molecular epidemiology of *Streptococcus uberis* clinical mastitis in dairy herds: Strain heterogeneity and transmission. J Clin Microbiol. 2016;54(1):68-74.
35. Delannoy CM, Crumlish M, Fontaine MC, *et al.* Human *Streptococcus agalactiae* strains in aquatic mammals and fish. BMC Microbiol. 2013;13:41.
36. Deluyker HA, Van Oye SN, Boucher JF. Factors affecting cure and somatic cell count after pirlimycin treatment of subclinical mastitis in lactating cows. J Dairy Sci. 2005;88(2):604-14.
37. Denis M, Parlane NA, Lacy-Hulbert SJ, *et al.* Bactericidal activity of macrophages against *Streptococcus uberis* is different in mammary gland secretions of lactating and drying off cows. Vet Immunol Immunopathol. 2006;114(1-2):111-20.

38. Denis M, Lacy-Hulbert SJ, Buddle BM, *et al. Streptococcus uberis*-specific T cells are present in mammary gland secretions of cows and can be activated to kill *S. uberis*. Vet Res Commun. 2011;35(3):145-56.

39. Devriese LA, Hommez J. Epidemiology of methicillin-resistant *Staphylococcus aureus* in dairy herds. Res Vet Sci. 1975;19(1):23-7.

40. Dimitracopoulos G, Kalkani-Boussiakou H, Papavassiliou J. Animal fecal carriership and biotypes of *Staphylococcus aureus*. Appl Environ Microbiol. 197;34(5):461-4.

41. Dogan B, Schukken YH, Santisteban C, *et al.* Distribution of serotypes and antimicrobial resistance genes among *Streptococcus agalactiae* isolates from bovine and human hosts. J Clin Microbiol. 2005;43(12):5899-906.

42. Dogan B, Rishniw M, Bruant G, *et al.* Phylogroup and *lpfA* influence epithelial invasion by mastitis associated *Escherichia coli*. Vet Microbiol. 2012;159(1-2):163-70.

43. Dohmen W, Neijenhuis F, Hogeveen H. Relationship between udder health and hygiene on farms with an automatic milking system. J Dairy Sci. 2010;93(9):4019-33.

44. Dohoo IR, Smith J, Andersen S, *et al.* Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. J Dairy Sci. 2011;94(1):250-61.

45. Döpfer D, Barkema HW, Lam TJ, *et al.* Recurrent clinical mastitis caused by *Escherichia coli* in dairy cows. J Dairy Sci. 1999;82(1):80-5.

46. Dorado-García A, Mevius DJ, Jacobs JJ, *et al.* Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands. J Antimicrob Chemother. 2016;71(12):3607-3619.

47. Down PM, Bradley AJ, Breen JE, *et al.* Current management practices and interventions prioritised as part of a nationwide mastitis control plan. Vet Rec. 2016;178(18):449.

48. Eckelkamp EA, Taraba JL, Akers KA, *et al.* Understanding compost bedded pack barns: Interactions among environmental factors, bedding characteristics, and udder health. Livestock Sci. 2016;190:35–42.

908 49. Edwards JP, O'Brien B, Lopez-Villalobos N, *et al.* Overmilking causes deterioration in teat-end
909 condition of dairy cows in late lactation. J Dairy Res. 2013;80(3):344-8.
910 50. Ericsson Unnerstad H, Lindberg A, Persson Waller K, *et al.* Microbial aetiology of acute clinical
911 mastitis and agent-specific risk factors. Vet Microbiol. 2009;137(1-2):90-7.
912 51. Erskine RJ. Vaccination strategies for mastitis. Vet Clin North Am Food Anim Pract.
913 2012;28(2):257-70.
914 52. Erskine RJ, Walker RD, Bolin CA, *et al.* Trends in antibacterial susceptibility of mastitis
915 pathogens during a seven-year period. J Dairy Sci. 2002;85(5):1111-8.
916 53. Falentin H, Rault L, Nicolas A, *et al.* Bovine teat microbiome analysis revealed reduced alpha
917 diversity and significant changes in taxonomic profiles in quarters with a history of mastitis.
918 Front Microbiol. 2016;7:480.
919 54. Favero S, Portilho FVR, Oliveira ACR *et al.* Longitudinal trends and associations between
920 compost bedding characteristics and bedding bacterial concentrations. J Agr Sci
921 2015;7(10):58-70.
922 55. Federal Drug Administration. 2012. 21 CFR Part 530. Federal Register 77(4)/Rules and
923 Regulations.
924 56. Foresight. The Future of Food and Farming (2011). Final Project Report. The Government
925 Office for Science, London.
926 57. Fox LK, Gay JM. Contagious mastitis. Vet Clin North Am Food Anim Pract. 1993;9(3):475-87.
927 58. Ganda EK, Bisinotto RS, Lima SF, *et al.* Longitudinal metagenomic profiling of bovine milk to
928 assess the impact of intramammary treatment using a third-generation cephalosporin. Sci
929 Rep.2016a;6:37565.
930 59. Ganda EK, Bisinotto RS, Decter DH, *et al.* Evaluation of an on-farm culture system (Accumast)
931 for fast identification of milk pathogens associated with clinical mastitis in dairy cows. PLoS
932 One. 2016b;11(5):e0155314.

933 60. Gelasakis AI, Mavrogianni VS, Petridis IG, *et al.* Mastitis in sheep--The last 10 years and the
934 future of research. Vet Microbiol. 2015;181(1-2):136-46.
935 61. Ghosh S, Nishat S, Andreana PR. Synthesis of an aminooxy derivative of the tetrasaccharide
936 repeating unit of *Streptococcus dysgalactiae* 2023 polysaccharide for a PS A1 conjugate
937 vaccine. J Org Chem. 2016;81(11):4475-84.
938 62. Gilchrist TL, Smith DG, Fitzpatrick JL, *et al.* Comparative molecular analysis of ovine and bovine
939 *Streptococcus uberis* isolates. J Dairy Sci. 2013;96(2):962-70.
940 63. Gitau GK, Bundi RM, Vanleeuwen J, *et al.* Evaluation of Petrifilms(TM) as a diagnostic test to
941 detect bovine mastitis organisms in Kenya. Trop Anim Health Prod. 2013;45(3):883-6.
942 64. Gonggrijp MA, Santman-Berends IM, Heuvelink AE, *et al.* Prevalence and risk factors for
943 extended-spectrum β-lactamase- and AmpC-producing *Escherichia coli* in dairy farms. J Dairy
944 Sci. 2016;99(11):9001-9013.
945 65. Green MJ, Bradley AJ, Medley GF, *et al.* Cow, farm, and management factors during the dry
946 period that determine the rate of clinical mastitis after calving. J Dairy Sci. 2007;90(8):3764-
947 76.
948 66. Griffioen K, Hop GE, Holstege MM, *et al.* Dutch dairy farmers' need for microbiological mastitis
949 diagnostics. J Dairy Sci. 2016;99(7):5551-61.
950 67. Gröhn YT, González RN, Wilson DJ, *et al.* Effect of pathogen-specific clinical mastitis on herd
951 life in two New York State dairy herds. Prev Vet Med. 2005;71(1-2):105-25.
952 68. Guarín JF, Baumberger C, Ruegg PL. Anatomical characteristics of teats and premilking
953 bacterial counts of teat skin swabs of primiparous cows exposed to different types of bedding.
954 J Dairy Sci. 2017;100(2):1436-1444.
955 69. Günther J, Czabanska A, Bauer I, *et al. Streptococcus uberis* strains isolated from the bovine
956 mammary gland evade immune recognition by mammary epithelial cells, but not of
957 macrophages. Vet Res. 2016;47:13.

70. Gurjar A, Gioia G, Schukken Y, *et al.* Molecular diagnostics applied to mastitis problems on dairy farms. Vet Clin North Am Food Anim Pract. 2012;28(3):565-76.

71. Halasa T, Huijps K, Østerås O, *et al.* Economic effects of bovine mastitis and mastitis management: a review. Vet Q. 2007;29(1):18-31.

72. Hertl JA, Schukken YH, Welcome FL, *et al.* Pathogen-specific effects on milk yield in repeated clinical mastitis episodes in Holstein dairy cows. J Dairy Sci. 2014a;97(3):1465-80.

73. Hertl JA, Schukken YH, Welcome FL, *et al.* Effects of pathogen-specific clinical mastitis on probability of conception in Holstein dairy cows. J Dairy Sci. 2014b;97(11):6942-54.

74. Higgins HM, Mouncey J, Nanjiani I, *et al.* Understanding how new evidence influences practitioners' beliefs regarding dry cow therapy: A Bayesian approach using probabilistic elicitation. Prev Vet Med. 2016;pii:S0167-5877(16)30312-9.

75. Higgins HM, Golding SE, Mouncey J, *et al.* Understanding veterinarians' prescribing decisions on antibiotic dry cow therapy. J Dairy Sci. 2017;100(4):2909-2916.

76. Hill AW. Pathogenicity of two strains of *Streptococcus uberis* infused into lactating and non-lactating bovine mammary glands. Res Vet Sci. 1988;45(3):400-4.

77. Hill AW, Finch JM, Field TR, *et al.* Immune modification of the pathogenesis of *Streptococcus uberis* mastitis in the dairy cow. FEMS Immunol Med Microbiol. 1994;8(2):109-17.

78. Hisaeda K, Arima H, Sonobe T, *et al.* Changes in acute-phase proteins and cytokines in serum and milk whey from dairy cows with naturally occurring peracute mastitis caused by *Klebsiella pneumoniae* and the relationship to clinical outcome. J Vet Med Sci.2011;73(11):1399-404.

79. Hogan J, Larry Smith K. Coliform mastitis. Vet Res. 2003;34(5):507-19.

80. Hogan JS, Wolf SL, Petersson-Wolfe CS. Bacterial counts in organic materials used as free-stall bedding following treatment with a commercial conditioner. J Dairy Sci. 2007;90(2):1058-62.

81. Holmes MA, Zadoks RN. Methicillin resistant *S. aureus* in human and bovine mastitis. J Mammary Gland Biol Neoplasia. 2011;16(4):373-82.

82. Holt KE, Wertheim H, Zadoks RN, *et al.* Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proc Natl Acad Sci U S A. 2015;112(27):E3574-81.
83. Hossain M, Egan SA, Coffey T, *et al.* Virulence related sequences; insights provided by comparative genomics of *Streptococcus uberis* of differing virulence. BMC Genomics. 2015;16:334.
84. Husfeldt AW, Endres MI, Salfer JA, *et al.* Management and characteristics of recycled manure solids used for bedding in Midwest freestall dairy herds. J Dairy Sci. 2012;95(4):2195-203.
85. Huxley JN, Greent MJ, Green LE, *et al.* Evaluation of the efficacy of an internal teat sealer during the dry period. J Dairy Sci. 2002;85(3):551-61.
86. Jánosi S, Szigeti G, Rátz F, *et al. Prototheca zopfii* mastitis in dairy herds under continental climatic conditions. Vet Q. 2001;23(2):80-3.
87. Jansen J, Lam TJ. The role of communication in improving udder health. Vet Clin North Am Food Anim Pract. 2012;28(2):363-79.
88. Jensen NE. Experimental bovine group-B streptococcal mastitis induced by strains of human and bovine origin. Nord Vet Med. 1982;34(12):441-50.
89. Johnson SD, Norcross NL. An experimental method of vaccination for a *Streptococcus agalactiae* infected herd. Cornell Vet. 1971;61(2):258-64.
90. Jørgensen HJ, Nordstoga AB, Sviland S, *et al. Streptococcus agalactiae* in the environment of bovine dairy herds—rewriting the textbooks? Vet Microbiol. 2016;184:64-72.
91. Kempf F, Slugocki C, Blum SE, *et al.* Genomic comparative study of bovine mastitis *Escherichia coli*. PLoS One. 2016;11(1):e0147954.
92. Klaas IC, Enevoldsen C, Ersbøll AK, *et al.* Cow-related risk factors for milk leakage. J Dairy Sci. 2005;88(1):128-36.

93. Koskinen MT, Holopainen J, Pyörälä S, *et al.* Analytical specificity and sensitivity of a real-time polymerase chain reaction assay for identification of bovine mastitis pathogens. J Dairy Sci. 2009;92(3):952-9.

94. Koskinen MT, Wellenberg GJ, Sampimon OC, *et al.* Field comparison of real-time polymerase chain reaction and bacterial culture for identification of bovine mastitis bacteria. J Dairy Sci. 2010;93(12):5707-15.

95. Kristula MA, Rogers W, Hogan JS, *et al.* Comparison of bacteria populations in clean and recycled sand used for bedding in dairy facilities. J Dairy Sci. 2005;88(12):4317-25.

96. Kristula MA, Dou Z, Toth JD, *et al.* Evaluation of free-stall mattress bedding treatments to reduce mastitis bacterial growth. J Dairy Sci. 2008;91(5):1885-92.

97. Lacy-Hulbert SJ, Hillerton JE. Physical characteristics of the bovine teat canal and their influence on susceptibility to streptococcal infection. J Dairy Res. 1995;62(3):395-404.

98. Lago A, Godden SM, Bey R, *et al.* The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. J Dairy Sci. 2011a;94(9):4441-56.

99. Lago A, Godden SM, Bey R, *et al.* The selective treatment of clinical mastitis based on on-farm culture results: II. Effects on lactation performance, including clinical mastitis recurrence, somatic cell count, milk production, and cow survival. J Dairy Sci. 2011b;94(9):4457-67.

100. Lanctôt S, Fustier P, Taherian AR, *et al.* Effect of intramammary infusion of chitosan hydrogels at drying-off on bovine mammary gland involution. J Dairy Sci. 2017 Mar;100(3):2269-2281.

101. Landin H, Mörk MJ, Larsson M, *et al.* Vaccination against *Staphylococcus aureus* mastitis in two Swedish dairy herds. Acta Vet Scand. 2015;57:81.

102. Leach KA, Archer SC, Breen JE, *et al.* Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms. Vet J. 2015;206(2):123-30.

103. Leigh JA. *Streptococcus uberis*: a permanent barrier to the control of bovine mastitis? Vet J. 1999;157(3):225-38.

104. Leslie KE, Petersson-Wolfe CS. Assessment and management of pain in dairy cows with clinical mastitis. Vet Clin North Am Food Anim Pract. 2012;28(2):289-305.

105. Lim GH, Leslie KE, Kelton DF, *et al.* Adherence and efficacy of an external teat sealant to prevent new intramammary infections in the dry period. J Dairy Sci. 2007;90(3):1289-300.

106. Lin J, Hogan JS, Smith KL. Growth responses of coliform bacteria to purified immunoglobulin G from cows immunized with ferric enterobactin receptor FepA. J Dairy Sci. 1999;82(1):86-92.

107. Liu G, Yin J, Barkema HW, *et al.* Development of a single-dose recombinant CAMP factor entrapping poly(lactide-co-glycolide) microspheres-based vaccine against *Streptococcus agalactiae*. Vaccine. 2017;35(9):1246-1253.

108. Locatelli C, Scaccabarozzi L, Pisoni G, *et al.* CTX-M1 ESBL-producing *Klebsiella pneumoniae* subsp. *pneumoniae* isolated from cases of bovine mastitis. J Clin Microbiol. 2010;48(10):3822-3.

109. Locatelli C, Cremonesi P, Bertocchi L, *et al.* Short communication: Methicillin-resistant *Staphylococcus aureus* in bulk tank milk of dairy cows and effect of swine population density. J Dairy Sci. 2016;99(3):2151-6.

110. Lopez-Benavides MG, Williamson JH, Pullinger GD, *et al.* Field observations on the variation of *Streptococcus uberis* populations in a pasture-based dairy farm. J Dairy Sci. 2007;90(12):5558-66.

111. Lopez-Benavides MG, Williamson JH, Lacy-Hulbert SJ, *et al.* Heifer teats sprayed in the dry period with an iodine teat sanitizer have reduced *Streptococcus uberis* teat-end contamination and less *Streptococcus uberis* intra-mammary infections at calving. Vet Microbiol. 2009;134(1-2):186-91.

112. Lundberg Å, Nyman A, Unnerstad HE, *et al.* Prevalence of bacterial genotypes and outcome of bovine clinical mastitis due to *Streptococcus dysgalactiae* and *Streptococcus uberis*. Acta Vet Scand. 2014;56:80.

113. Lyhs U, Kulkas L, Katholm J, *et al. Streptococcus agalactiae* serotype IV in humans and cattle, northern Europe. Emerg Infect Dis. 2016;22(12):2097-2103.
114. Mahmmod YS, Klaas IC, Katholm J, *et al.* Molecular epidemiology and strain-specific characteristics of *Streptococcus agalactiae* at the herd and cow level. J Dairy Sci. 2015;98(10):6913-24.
115. Makovec JA, Ruegg PL. Antimicrobial resistance of bacteria isolated from dairy cow milk samples submitted for bacterial culture: 8,905 samples (1994-2001). J Am Vet Med Assoc. 2003;222(11):1582-9.
116. Maloney, T. 2002. Management of Hispanic employees on New York dairy farms: a survey of farm managers. *pp.* 67-78 in: The Dynamics of Hired Farm Labour: Constraints and Community Responses. Findeis JL, Vandeman AM, Larson JM, *et al.* (Eds). CABI Publishing.
117. Mansion-de Vries EM, Knorr N, Paduch JH, *et al.* A field study evaluation of Petrifilm™ plates as a 24-h rapid diagnostic test for clinical mastitis on a dairy farm. Prev Vet Med. 2014;113(4):620-4.
118. McCarron JL, Keefe GP, McKenna SL, *et al.* Laboratory evaluation of 3M Petrifilms and University of Minnesota Bi-plates as potential on-farm tests for clinical mastitis. J Dairy Sci. 2009;92(5):2297-305.
119. McDougall S, Agnew KE, Cursons R, *et al.* Parenteral treatment of clinical mastitis with tylosin base or penethamate hydriodide in dairy cattle. J Dairy Sci. 2007a;90(2):779-89.
120. McDougall S, Arthur DG, Bryan MA, *et al.* Clinical and bacteriological response to treatment of clinical mastitis with one of three intramammary antibiotics. N Z Vet J. 2007b;55(4):161-70.
121. Muellner P, Zadoks RN, Perez AM, *et al.* The integration of molecular tools into veterinary and spatial epidemiology. Spat Spatiotemporal Epidemiol. 2011;2(3):159-71.
122. Munoz MA, Zadoks RN. Short communication: Patterns of fecal shedding of *Klebsiella* by dairy cows. J Dairy Sci. 2007;90(3):1220-4.

123. Munoz MA, Ahlström C, Rauch BJ, *et al.* Fecal shedding of *Klebsiella pneumoniae* by dairy cows. J Dairy Sci. 2006;89(9):3425-30.

124. Munoz MA, Welcome FL, Schukken YH, *et al.* Molecular epidemiology of two *Klebsiella pneumoniae* mastitis outbreaks on a dairy farm in New York State. J Clin Microbiol. 2007;45(12):3964-71.

125. Munoz MA, Bennett GJ, Ahlström C, *et al*. Cleanliness scores as indicator of *Klebsiella* exposure in dairy cows. J Dairy Sci. 2008;91(10):3908-16.

126. Mweu MM, Nielsen SS, Halasa T, *et al.* Annual incidence, prevalence and transmission characteristics of *Streptococcus agalactiae* in Danish dairy herds. Prev Vet Med. 2012;106(3-4):244-50.

127. Mweu MM, Nielsen SS, Halasa T, *et al.* Spatiotemporal patterns, annual baseline and movement-related incidence of *Streptococcus agalactiae* infection in Danish dairy herds: 2000-2009. Prev Vet Med. 2014;113(2):219-30.

128. Oikonomou G, Machado VS, Santisteban C, *et al.* Microbial diversity of bovine mastitic milk as described by pyrosequencing of metagenomics 16s rDNA. PLoS One. 2012;7(10):e47671.

129. Olde Riekerink RG, Barkema HW, Stryhn H. The effect of season on somatic cell count and the incidence of clinical mastitis. J Dairy Sci. 2007;90(4):1704-15.

130. Oliveira L, Hulland C, Ruegg PL. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. J Dairy Sci. 2013;96(12):7538-49.

131. Oliver SP, Gillespie BE, Headrick SJ, *et al.* Efficacy of extended ceftiofur intramammary therapy for treatment of subclinical mastitis in lactating dairy cows. J Dairy Sci. 2004;87(8):2393-400.

132. O'Neill. 2016. Tackling Drug-Resistant Infections Globally: final report and recommendations. https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf

133. Osborne AD, Armstrong K, Catrysse NH, *et al.* An outbreak of *Pseudomonas* mastitis in dairy cows. Can Vet J. 1981;22(7):215-6.

134. Østerås O, Edge VL, Martin SW. Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. J Dairy Sci. 1999;82(6):1221-31.
135. Ostrum PG, Thomas MJ, Zadoks RN. Dried manure solids for freestall bedding: Experiences from a Northeast dairy. Pages 149–156 in Proceedings of the NMC Annual Meeting, 2008. National Mastitis Council (NMC), Madison, WI.
136. Osumi T, Kishimoto Y, Kano R, *et al. Prototheca zopfii* genotypes isolated from cow barns and bovine mastitis in Japan. Vet Microbiol. 2008;131(3-4):419-23.
137. Owens WE, Oliver SP, Gillespie BE, *et al.* Role of horn flies (*Haematobia irritans*) in *Staphylococcus aureus*-induced mastitis in dairy heifers. Am J Vet Res. 1998;59(9):1122-4.
138. Paduch JH, Mohr E, Krömker V. The association between teat end hyperkeratosis and teat canal microbial load in lactating dairy cattle. Vet Microbiol. 2012;158(3-4):353-9.
139. Paduch JH, Mohr E, Krömker V. The association between bedding material and the bacterial counts of *Staphylococcus aureus*, *Streptococcus uberis* and coliform bacteria on teat skin and in teat canals in lactating dairy cattle. J Dairy Res. 2013;80(2):159-64
140. Pereira UP, Oliveira DG, Mesquita LR, *et al.* Efficacy of *Staphylococcus aureus* vaccines for bovine mastitis: a systematic review. Vet Microbiol. 2011;148(2-4):117-24.
141. Perez-Casal J, Prysliak T, Potter AA. A GapC chimera retains the properties of the *Streptococcus uberis* wild-type GapC protein. Protein Expr Purif. 2004;33(2):288-96.
142. Perrig MS, Ambroggio MB, Buzzola FR, *et al.* Genotyping and study of the pauA and sua genes of *Streptococcus uberis* isolates from bovine mastitis. Rev Argent Microbiol. 2015;47(4):282-94.
143. Persson Waller K, Westermark T, Ekman T, *et al.* Milk leakage--an increased risk in automatic milking systems. J Dairy Sci. 2003;86(11):3488-97.
144. Petersson-Wolfe CS, Wolf SL, Hogan JS. Experimental challenge of bovine mammary glands with *Enterococcus faecium* during early and late lactation. J Dairy Sci. 2009;92(7):3158-64.

145. Pighetti GM, Elliott AA. Gene polymorphisms: the keys for marker assisted selection and unraveling core regulatory pathways for mastitis resistance. J Mammary Gland Biol Neoplasia. 2011;16(4):421-32.

146. Plumed-Ferrer C, Uusikylä K, Korhonen J, *et al.* Characterization of *Lactococcus lactis* isolates from bovine mastitis. Vet Microbiol. 2013;167(3-4):592-9.

147. Pomeroy B, Gurjar A, Sipka A, *et al.* Intramammary immunization with ultraviolet-killed *Escherichia coli* shows partial protection against late gestation intramammary challenge with a homologous strain. J Dairy Sci. 2016;99(11):9014-9026. d

148. Prado ME, Almeida RA, Ozen C, *et al.* Vaccination of dairy cows with recombinant *Streptococcus uberis* adhesion molecule induces antibodies that reduce adherence to and internalization of *S. uberis* into bovine mammary epithelial cells. Vet Immunol Immunopathol. 2011;141(3-4):201-8.

149. Price JR, Cole K, Bexley A, *et al.* Transmission of *Staphylococcus aureus* between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. Lancet Infect Dis. 2017;17(2):207-214..

150. Proietto RL, Hinckley LS, Fox LK, *et al.* Evaluation of a clay-based acidic bedding conditioner for dairy cattle bedding. J Dairy Sci. 2013;96(2):1044-53.

151. Pyörälä S, Hovinen M, Simojoki H, *et al.* Acute phase proteins in milk in naturally acquired bovine mastitis caused by different pathogens. Vet Rec. 2011;168(20):535.

152. Quesnell RR, Klaessig S, Watts JL, *et al.* Bovine intramammary *Escherichia coli* challenge infections in late gestation demonstrate a dominant antiinflammatory immunological response. J Dairy Sci. 2012;95(1):117-26.

153. Rabiee AR, Lean IJ. The effect of internal teat sealant products (Teatseal and Orbeseal) on intramammary infection, clinical mastitis, and somatic cell counts in lactating dairy cows: a meta-analysis. J Dairy Sci. 2013;96(11):6915-31.

154. Randall L, Heinrich K, Horton R, *et al.* Detection of antibiotic residues and association of cefquinome residues with the occurrence of Extended-Spectrum β-Lactamase (ESBL)-producing bacteria in waste milk samples from dairy farms in England and Wales in 2011. Res Vet Sci. 2014;96(1):15-24.

155. Reyher KK, Haine D, Dohoo IR, *et al.* Examining the effect of intramammary infections with minor mastitis pathogens on the acquisition of new intramammary infections with major mastitis pathogens--a systematic review and meta-analysis. J Dairy Sci. 2012;95(11):6483-502.

156. Richards VP, Zadoks RN, Pavinski Bitar PD, *et al.* Genome characterization and population genetic structure of the zoonotic pathogen, *Streptococcus canis*. BMC Microbiol. 2012;12:293.

157. Richards VP, Lefébure T, Pavinski Bitar PD, *et al.* Genome based phylogeny and comparative genomic analysis of intra-mammary pathogenic *Escherichia coli*. PLoS One. 2015;10(3):e0119799.

158. Rindsig RB, Rodewald RG, Smith AR, *et al.* Complete versus selective dry cow therapy for mastitis control. J Dairy Sci. 1978;61(10):1483-97.

159. Roberson JR. Treatment of clinical mastitis. Vet Clin North Am Food Anim Pract. 2012;28(2):271-88.

160. Roberson JR, Fox LK, Hancock DD, *et al.* Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. J Dairy Sci. 1994;77(11):3354-64.

161. Rodrigues MX, Lima SF, Higgins CH, *et al.* The *Lactococcus* genus as a potential emerging mastitis pathogen group: A report on an outbreak investigation. J Dairy Sci. 2016;99(12):9864-9874.

162. Rowbotham RF, Ruegg PL. Bacterial counts on teat skin and in new sand, recycled sand, and recycled manure solids used as bedding in freestalls. J Dairy Sci. 2016;99(8):6594-608.

163. Saini V, McClure JT, Léger D, *et al.* Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. J Dairy Sci. 2012;95(8):4319-32.

164. Sampimon O, Barkema HW, Berends I, *et al.* Prevalence of intramammary infection in Dutch dairy herds. J Dairy Res. 2009a;76(2):129-36.

165. Sampimon OC, Zadoks RN, De Vliegher S, *et al.* Performance of API Staph ID 32 and Staph-Zym for identification of coagulase-negative staphylococci isolated from bovine milk samples. Vet Microbiol. 2009;136(3-4):300-5.

166. Santman-Berends IM, Gonggrijp MA, Hage JJ, *et al.* Prevalence and risk factors for extended-spectrum β-lactamase or AmpC-producing *Escherichia coli* in organic dairy herds in the Netherlands. J Dairy Sci. 2017;100(1):562-571.

167. Schabauer L, Wenning M, Huber I, *et al.* Novel physico-chemical diagnostic tools for high throughput identification of bovine mastitis associated gram-positive, catalase-negative cocci. BMC Vet Res. 2014;10:156.

168. Scherpenzeel CG, den Uijl IE, van Schaik G, *et al.* Evaluation of the use of dry cow antibiotics in low somatic cell count cows. J Dairy Sci. 2014;97(6):3606-14.

169. Scherpenzeel CG, Tijs SH, den Uijl IE, *et al.* Farmers' attitude toward the introduction of selective dry cow therapy. J Dairy Sci. 2016;99(10):8259-66.

170. Schukken YH, Vanvliet J, Vandegeer D, *et al.* A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. J Dairy Sci. 1993;76(10):2925-30.

171. Schukken YH, Bennett GJ, Zurakowski MJ, *et al.* Randomized clinical trial to evaluate the efficacy of a 5-day ceftiofur hydrochloride intramammary treatment on nonsevere gram-negative clinical mastitis. J Dairy Sci. 2011a;94(12):6203-15.

172. Schukken YH, Günther J, Fitzpatrick J, *et al.* Host-response patterns of intramammary infections in dairy cows. Vet Immunol Immunopathol. 2011b;144(3-4):270-89.

173. Schukken Y, Chuff M, Moroni P, *et al.* The "other" gram-negative bacteria in mastitis: *Klebsiella*, *Serratia*, and more. Vet Clin North Am Food Anim Pract. 2012;28(2):239-56.

174. Schukken YH, Zurakowski MJ, Rauch BJ, *et al.* Noninferiority trial comparing a first-generation cephalosporin with a third-generation cephalosporin in the treatment of nonsevere clinical mastitis in dairy cows. J Dairy Sci. 2013;96(10):6763-74.

175. Schukken YH, Bronzo V, Locatelli C, *et al.* Efficacy of vaccination on *Staphylococcus aureus* and coagulase-negative staphylococci intramammary infection dynamics in 2 dairy herds. J Dairy Sci. 2014;97(8):5250-64.

176. Seegers H, Fourichon C, Beaudeau F. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet Res. 2003;34(5):475-91.

177. Shearn MF, Hillerton JE. Hyperkeratosis of the teat duct orifice in the dairy cow. J Dairy Res. 1996;63(4):525-32.

178. Sheet OH, Grabowski NT, Klein G, *et al.* Development and validation of a loop mediated isothermal amplification (LAMP) assay for the detection of *Staphylococcus aureus* in bovine mastitis milk samples. Mol Cell Probes. 2016;30(5):320-325.

179. Smith KL, Todhunter DA, Schoenberger PS. Environmental mastitis: cause, prevalence, prevention. J Dairy Sci. 1985;68(6):1531-53.

180. Smith KL, Hogan JS, Weiss WP. Dietary vitamin E and selenium affect mastitis and milk quality. J Anim Sci. 1997;75(6):1659-65.

181. Smith TH, Fox LK, Middleton JR. Outbreak of mastitis caused by one strain of *Staphylococcus aureus* in a closed dairy herd. J Am Vet Med Assoc 1998;212:553–556.

182. Smith GW, Lyman RL, Anderson KL. Efficacy of vaccination and antimicrobial treatment to eliminate chronic intramammary *Staphylococcus aureus* infections in dairy cattle. J Am Vet Med Assoc. 2006;228(3):422-5.

183. Sommerhäuser J, Kloppert B, Wolter W, *et al.* The epidemiology of *Staphylococcus aureus* infections from subclinical mastitis in dairy cows during a control programme. Vet Microbiol. 2003;96(1):91-102.

184. Sorter DE, Kester HJ, Hogan JS. Short communication: Bacterial counts in recycled manure solids bedding replaced daily or deep packed in freestalls. J Dairy Sci. 2014;97(5):2965-8.
185. Suojala L, Kaartinen L, Pyörälä S. Treatment for bovine *Escherichia coli* mastitis - an evidence-based approach. J Vet Pharmacol Ther. 2013;36(6):521-31.
186. Suriyasathaporn W, Heuer C, Noordhuizen-Stassen EN, *et al.* Hyperketonemia and the impairment of udder defense: a review. Vet Res. 2000;31(4):397-412.
187. Tassi R, McNeilly TN, Fitzpatrick JL, *et al.* Strain-specific pathogenicity of putative host-adapted and nonadapted strains of *Streptococcus uberis* in dairy cattle. J Dairy Sci. 201;96(8):5129-45.
188. Tassi R, McNeilly TN, Sipka A, *et al.* Correlation of hypothetical virulence traits of two *Streptococcus uberis* strains with the clinical manifestation of bovine mastitis. Vet Res. 2015;46:123.
189. Tavakol M, Riekerink RG, Sampimon OC, *et al.* Bovine-associated MRSA ST398 in the Netherlands. Acta Vet Scand. 2012;54:28.
190. Tipples R, Trafford S. 2011. Where will the milkers come from? A future employment conundrum for New Zealand's largest export industry. Employment Relations Record 11;43-61.
191. Tomita T, Meehan B, Wongkattiya N, *et al.* Identification of *Streptococcus uberis* multilocus sequence types highly associated with mastitis. Appl Environ Microbiol. 2008;74(1):114-24.
192. Trang NH, Nga TV, Campbell JI, *et al.* The characterization of ESBL genes in *Escherichia coli* and *Klebsiella pneumoniae* causing nosocomial infections in Vietnam. J Infect Dev Ctries. 2013;7(12):922-8.
193. Tremetsberger L, Leeb C, Winckler C. Animal health and welfare planning improves udder health and cleanliness but not leg health in Austrian dairy herds. J Dairy Sci. 2015;98(10):6801-11.
194. Van den Heever LW, Giesecke WH. Experimental induction of bovine mastitis with human strains of group B streptococci (*Streptococcus agalactiae*). J S Afr Vet Assoc. 1980;51(2):107-9.

195. Vanderhaeghen W, Cerpentier T, Adriaensen C, *et al.* Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. Vet Microbiol. 2010;144(1-2):166-71.

196. Vanderhaeghen W, Piepers S, Leroy F, *et al.* Identification, typing, ecology and epidemiology of coagulase negative staphylococci associated with ruminants. Vet J. 2015;203(1):44-51.

197. Viguier C, Arora S, Gilmartin N, *et al.* Mastitis detection: current trends and future perspectives. Trends Biotechnol. 2009;27(8):486-93.

198. Viora L, Graham EM, Mellor DJ, *et al.* Evaluation of a culture-based pathogen identification kit for bacterial causes of bovine mastitis. Vet Rec. 2014;175(4):89.

199. Virgin JE, Van Slyke TM, Lombard JE, *et al.* Short communication: methicillin-resistant *Staphylococcus aureus* detection in US bulk tank milk. J Dairy Sci. 2009;92(10):4988-91.

200. Wang D, Liu Y. Development of Primer Sets for Loop-Mediated Isothermal Amplification that Enables Rapid and Specific Detection of *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Streptococcus agalactiae.* Int J Environ Res Public Health. 2015;12(6):5735-42.

201. Wedlock DN, Buddle BM, Williamson J, *et al.* Dairy cows produce cytokine and cytotoxic T cell responses following vaccination with an antigenic fraction from *Streptococcus uberis*. Vet Immunol Immunopathol. 2014;160(1-2):51-60.

202. Wenz JR, Barrington GM, Garry FB, *et al.* Bacteremia associated with naturally occurring acute coliform mastitis in dairy cows. J Am Vet Med Assoc. 2001;219(7):976-81.

203. Wilson DJ, Mallard BA, Burton JL, *et al.* Milk and serum J5-specific antibody responses, milk production change, and clinical effects following intramammary *Escherichia col*i challenge for J5 vaccinate and control cows. Clin Vaccine Immunol. 2007;14(6):693-9.

204. Wilson DJ, Grohn YT, Bennett GJ, *et al.* Milk production change following clinical mastitis and reproductive performance compared among J5 vaccinated and control dairy cattle. J Dairy Sci. 2008;91(10):3869-79.

205. World Health Organization. 2015. Global Action Plan on Antimicrobial Resistance. ISBN 978 92 4 150976 3.

206. World Health Organization. 2017a. Global priority list of antibiotics-resistant bacteria to guide research, discovery, and development of new antibiotics. http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1.

207. World Health Organization. 2017b. Critically important antimicrobials for human medicine. 5th revision. ISBN: 978-92-4-151222-0.

208. Yancey RJ. Vaccines and diagnostic methods for bovine mastitis: fact and fiction. Adv Vet Med. 1999;41:257-73.

209. Yeruham I, Schwimmer A, Brami Y. Epidemiological and bacteriological aspects of mastitis associated with yellow-jacket wasps (*Vespula germanica*) in a dairy cattle herd. J Vet Med B Infect Dis Vet Public Health. 2002;49(10):461-3.

210. Zadoks RN. Sources and epidemiology of *Streptococcus uberis*, with special emphasis on mastitis in dairy cattle. Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2007;2(30).

211. Zadoks RN, Watts JL. Species identification of coagulase-negative staphylococci: genotyping is superior to phenotyping. Vet Microbiol. 2009;134(1-2):20-8.

212. Zadoks R, van Leeuwen W, Barkema H, *et al.*Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. J Clin Microbiol. 2000;38(5):1931-9.

213. Zadoks RN, Allore HG, Barkema HW, *et al.* Analysis of an outbreak of *Streptococcus uberis* mastitis. J Dairy Sci. 2001;84(3):590-9.

214. Zadoks RN, Gillespie BE, Barkema HW, *et al.* Clinical, epidemiological and molecular characteristics of *Streptococcus uberis* infections in dairy herds. Epidemiol Infect. 2003;130(2):335-49.

215. Zadoks RN, Tikofsky LL, Boor KJ. Ribotyping of *Streptococcus uberis* from a dairy's environment, bovine feces and milk. Vet Microbiol. 2005;109(3-4):257-65.

216. Zadoks RN, Middleton JR, McDougall S, *et al.* Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. J Mammary Gland Biol Neoplasia. 2011a;16(4):357-72.

217. Zadoks RN, Griffiths HM, Munoz MA, *et al.* Sources of *Klebsiella* and *Raoultella* species on dairy farms: be careful where you walk. J Dairy Sci. 2011b ;94(2):1045-51.

218. Zdanowicz M, Shelford JA, Tucker CB, *et al.* Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. J Dairy Sci. 2004;87(6):1694-701.

219. Zehner MM, Farnsworth RJ, Appleman RD, *et al.* Growth of environmental mastitis pathogens in various bedding materials. J Dairy Sci. 1986;69(7):1932-41.

220. Zoche-Golob V, Haverkamp H, Paduch JH, *et al.* Longitudinal study of the effects of teat condition on the risk of new intramammary infections in dairy cows. J Dairy Sci. 2015;98(2):910-7.

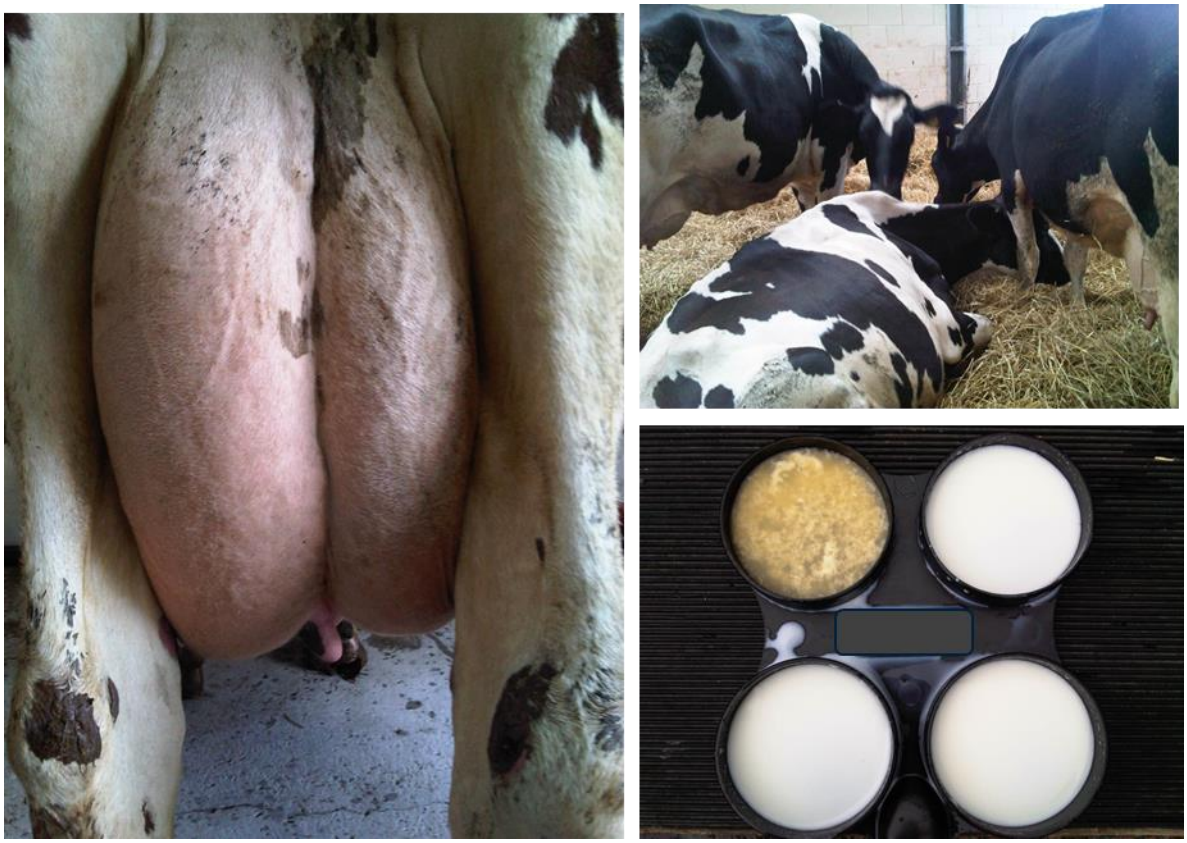

Figure 1. Severe clinical mastitis characterized by abnormalities in milk (bottom right panel), mammary gland (left panel) and behaviour (top right panel) due to *Streptococcus uberis* infection. Photos: RN Zadoks.

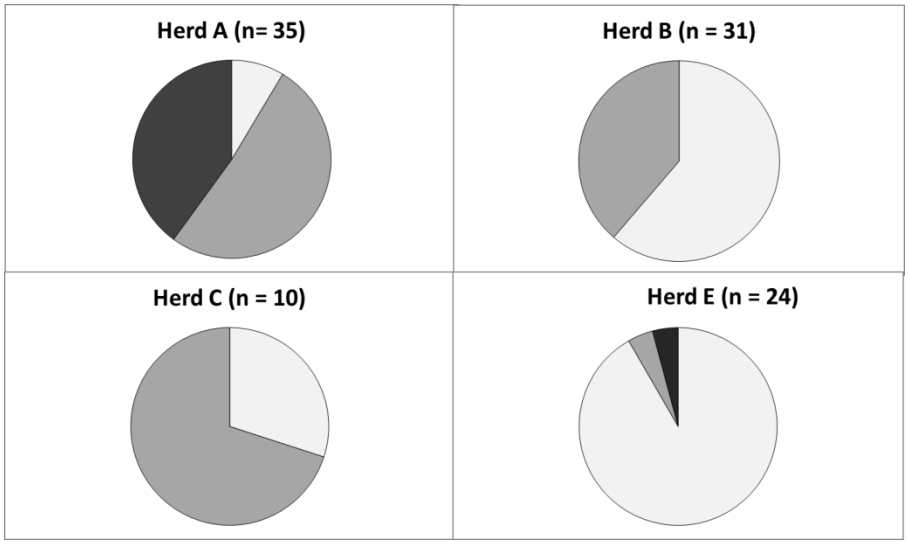

Figure 2. Herd-specific proportional distribution of mild to moderate cases of mastitis attributed to gram-negative pathogens. Black = *Enterobacter cloacae*; Off-white = *Escherichia coli*; Grey = *Klebsiella* spp. Number (n) of cases per herd shown in brackets. (Data: Schukken et al., 2011a; Herd D (n = 4) not shown).

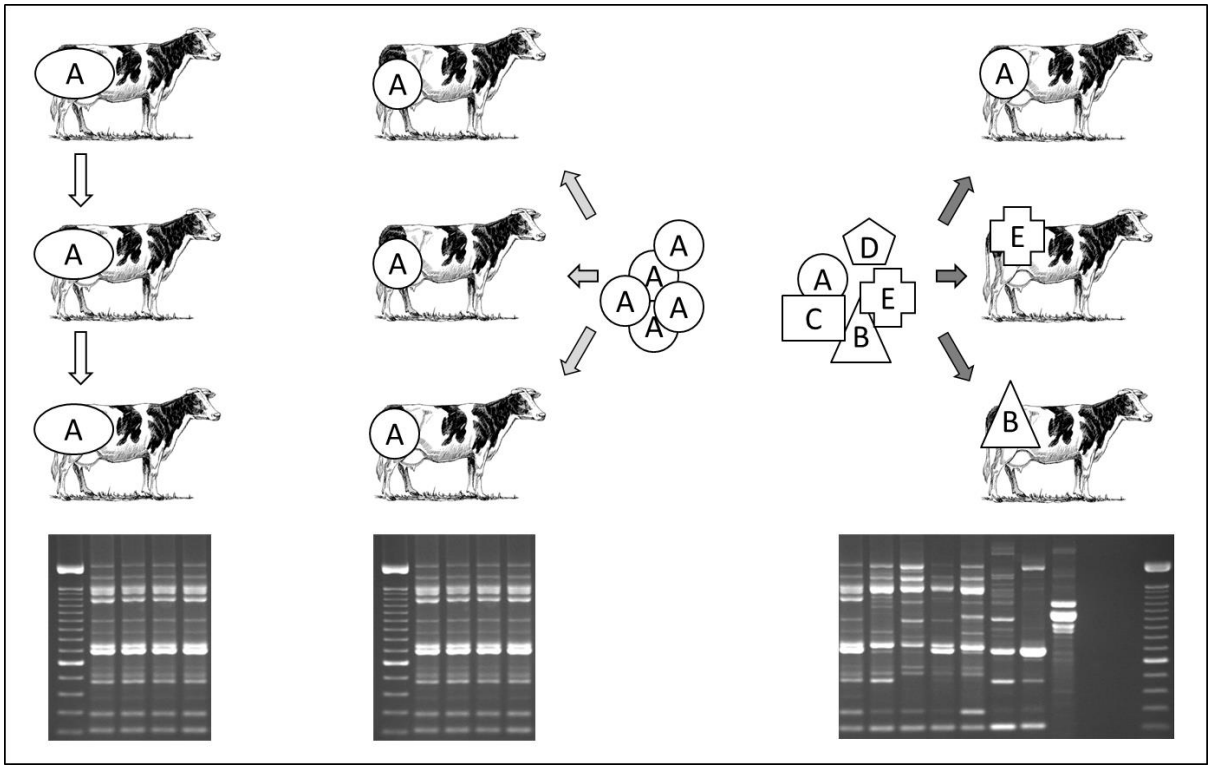

Figure 3. Modes of transmission (Left: contagious; Centre: environmental point source; Right: heterogeneous environmental source) and resultant patterns of strain distribution (Left, Centre: homogeneous; Right: heterogeneous), demonstrating that strain heterogeneity is proof of environmental origin of mastitis pathogens but homogeneity is not proof of contagious transmission.

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. Photos: 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. Photos: RN Zadoks.