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Udder health on Bavarian dairy farms
– a cross-sectional study

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Meiner lieben Familie

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ABBREVIATIONS

AMS	Automatic milking system
BC	Bacterial count
BPV	Bovine papillomavirus
BTBC	Bulk tank bacterial count
BTSCC	Bulk tank somatic cell count
Cfu	Colony forming unit
CI	Confidence interval
CM	Clinical mastitis
CMT	California mastitis test
CNS	Coagulase-negative staphylococci
DHIA	Dairy Herd Improvement Association
DIM	Days in milk
DVG	German Veterinary Medical Society (Deutsche Veterinärmedizinische Gesellschaft)
E.	Escherichia
e.g.	Example given
FAWC	Farm Animal Welfare Council
IDF	International Dairy Federation
i.e.	Id est (that is)
IMI	Intramammary infection
IQR	Interquartile range
LHS	Leg hygiene score
LKV	Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V. (Landeskontrollverband)
LPS	Lipopolysaccharide
MALDI-TOF	Matrix-assisted laser desorption/ionization - time of flight
Max	Maximum
Min	Minimum
NMC	National Mastitis Council
NMPF	National Milk Producers Federation
OIE	World Organisation for Animal Health
PMN	Polymorphonuclear neutrophils
RohMilch-GütV	Raw Milk Quality Ordinance (Rohmilchgüteverordnung)
OR	Odds ratio
S.	*Staphylococcus*
SCC	Somatic cell counts
Str.	*Streptococcus*
SUBM	Subclinical mastitis
TGD	Bavarian animal health services (Tiergesundheitsdienst Bayern e.V.)
UCD	Udder cleft dermatitis
UE	Udder edema
UHS	Udder hygiene score
UTD	Udder thigh dermatitis
WHO	Word Health Organization

I. INTRODUCTION

Udder health is of utmost importance for animal welfare, milk quality and economic success of dairy farms. Udder diseases and their associated risk factors must be addressed with appropriate preventive measures. In order to establish these, as well as to identify knowledge gaps and future research projects, awareness of the current prevalence of these diseases is crucial. Prevalence studies provide a data base about the current situation and information on associations with potential risk or sparring factors.

In Germany, few studies on the prevalence of mastitis pathogens exist. Often, these are data from diagnostic laboratories, which give a good overview of the distribution of laboratory submissions, but not of the overall situation. In most cases, samples are a mixture of individual submissions (mostly diseased animals) and herd examinations (often problem herds).
In addition, at the time of the investigation no studies on udder diseases other than mastitis in Germany were available – with the exception of two studies on udder thigh dermatitis (Sigmund et al., 1980; Sickinger et al., 2022).

Therefore, the aim of the present work was to determine the prevalence of the udder diseases: teat warts, udder edema (UE), udder thigh dermatitis (UTD), and udder cleft dermatitis (UCD). Analyses were performed for each of these diseases to determine potential risk factors at both herd- and cow-level (Publication I).
Furthermore, the prevalence of mastitis, more precisely the mastitis pathogens, was assessed and associations of management practices with the presence at herd-level for the four most common pathogens, namely coagulase-negative staphylococci (CNS), *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* were identified (Publication II).

II. LITERATURE REVIEW

1. Definition of udder health

Udder health is defined as "the condition of the bovine mammary gland which, on the basis of clinical examination, including secretion characteristics, as well as bacteriological, cytological and physico-chemical findings, corresponds to the physiological standard values" (Wiesner and Ribbeck, 1991).

The assessment of udder health can be done at the individual animal and herd-level. At the level of the individual cow, visible pathological changes of the secretion and udder tissue, as they occur in clinical mastitis (CM), can be detected by clinical examination. Nonvisible pathological changes of the cows' udder or in the milk, such as elevated somatic cell counts (SCC) in the context of a subclinical mastitis (SUBM), require further diagnostic measures. Somatic cells in milk are predominantly composed of leucocytes (macrophages, lymphocytes, and polymorphonuclear neutrophilic leucocytes) and approximately 1-2% epithelial cells (Schultz, 1977; Burvenich et al., 2009). Mostly in the case of inflammation (SUBM or CM) or local trauma, but also due to other influences such as lactation stage or lactation number, the cell count can increase considerably (Winter, 2009). Thus, the determination of the SCC in the milk can be used to indicate an intramammary infection (IMI). The physiological threshold for the SCC for milk in a quarter in a cow is usually under 100,000 cells/mL according to the National Mastitis Council (NMC, 2001). In Germany, this limit is also considered standard as defined by the German Veterinary Medical Society (Deutsche Veterinärmedizinische Gesellschaft, DVG, 2012). Thresholds at which an IMI can be expected are 200,000 cells/mL for quarter milk samples (Dohoo and Leslie, 1991; NMC, 2001; Petzer et al., 2017) and 150,000 cells/mL for composite milk samples (Petzer et al., 2017).

There are associations for controlling the performance and milk quality as well as the health status of individual cows and herds. In the USA, this is done by the Dairy Herd Improvement Association (DHIA), the German equivalent is the Landeskontrollverband (LKV) of the respective federal states. When (voluntarily) joining, farmers participate in monthly milk test sampling. On one hand, farmers receive data on milk yield, fat and protein concentrations in milk, fat-protein ratio, SCC, urea concentration, and more on the individual cow's milk. On the other hand, the LKV provides an evaluation of the entire herd: average performance, an average cell count, and more.

Generally, on a herd-level, the bulk tank somatic cell count (BTSCC) is an essential tool to assess the udder health status. A BTSCC of 200,000 cells/mL indicates that up to 15% of cows have an infection in one or more quarters, an increase of every 100,000 cells/mL implies that the infection rate raises by 8 to 10% (Eberhart et al., 1982). It is important to note here, that since farmers do not add the milk of cows known to have mastitis to the bulk tank, the BTSCC represents a biased value and therefore does not fully reflect herd status.

In addition to the BTSCC, the bulk tank bacterial count (BTBC) can be determined. The latter, however, not only displays the pathogens that enter the tank due to udder infections, but also contamination due to dirty teats or udders, hygiene problems in the milking equipment or inadequate cooling of the tank milk. Pathogen differentiation can be performed to identify the cause (O'Connell et al., 2016).

The determination of the two values (BTSCC and BTBC) primarily provide conclusions about the presence of mastitis in the herd. To detect herd problems of other udder diseases (e.g., udder skin diseases), the cows must be examined individually in the barn.

2. Importance of udder health

Udder health is an important aspect of dairy farming in terms of economics, for food safety and for animal welfare. The importance will be explained in the following sections in more detail.

2.1. Economy

Udder health plays a major financial role in the dairy industry. Mastitis, the inflammation of the mammary gland, is the most expensive disease for the dairy industry. A study from The Netherlands estimated annual mastitis costs at an average of 240€ per cow, including preventive and failure costs (van Soest et al., 2016). The costs are very complex and often underestimated by farmers (Huijps et al., 2008). In addition to the losses due to the drop in milk yield caused by the disease, there are also veterinary, laboratory and medication costs, waiting times, an additional workload, and in the worst case, the cow has to be involuntarily culled and replaced. The costs arising from the infection of healthy cows by the ones suffering from CM should not be underestimated (Down et al., 2013), this will also apply to SUBM. Furthermore, there are the costs for preventive measures such as vaccinations or, for example, dipping products or paper towels for pre-cleaning of the udder.

If the BTSCC and the BTBC are generally too high, deductions are also made when the milk is delivered. In Germany, according to the Milk Quality Ordinance, the BTSCC must be below 400,000 cells/mL (Rohmilchgüteverordnung, RohmilchGütV). In addition, according to Regulation (EC) No 853/2004 (European Commission, 2004), milk may only be obtained from cows without wounds on the udder that may affect the milk, and milk from cows with clinical udder diseases may only be admitted for consumption in agreement with a veterinarian.

Although there are no studies on udder diseases other than mastitis in terms of cost, for example veterinary costs, they may also be incurred for diseases such as UCD or UTD. For example, UCD has been associated with the incidence of mastitis (Persson Waller et al., 2014), with the aforementioned costs. In addition, if the ulcer perforates the mammary vein, the cow may bleed to death (Bouma et al., 2016).

2.2. Food safety, food quality and consumer protection

Poor udder health will not only affect costumer safety, but also food quality, processing and shelf life of milk and dairy products as well.

As an example, heat-treated milk from milk with high SCC has a shorter average shelf life than milk produced from milk with low SCC (Ma et al., 2000). The increased level of free fatty acids and casein hydrolysis causes pasteurized milk with high cell counts to show (primarily taste) quality impairments after a short time (14 to 21 days). Moreover, milk with high SCC also has negative effects in cheese production. In addition to a generally lower cheese yield (Barbano et al., 1990), the curd contains more moisture and is less firm (Ali et al., 1980) as well as there are increased fat content and casein losses in the whey (Politis and Ng-Kwai-Hang, 1988).

In raw milk and raw milk products, besides harmless bacteria, foodborne pathogens can be present (e.g., *Listeria* spp., *Campylobacter* spp.). However, apart from affecting the processing and shelf life of milk (products), the most important factor is food safety for the consumer.

In addition to pathogens that enter milk due to contamination during the processing, mastitis pathogens in raw milk can also be harmful to the consumer. Under certain conditions, such as inadequate refrigeration, *Staphylococcus* (*S.*)

aureus can form enterotoxins that can cause foodborne diseases (McMillan et al., 2016; Artursson et al., 2018). For example, there was a staphylococcal enterotoxin food poisoning outbreak in Switzerland in 2014 that occurred after consumption of raw milk cheese containing *S. aureus* strains, which were exclusively associated with a high within-herd prevalence of *S. aureus* mastitis (Johler et al., 2015).

Another risk factor in milk are antibiotic residues that can be found in raw milk due to mastitis treatment and subsequent lack of or inadequate milk discard. This might lead to allergies in the consumer (Ormerod et al., 1986). In addition, widespread antibiotic use may lead to an increased antimicrobial resistance. Bacteria with transmissible antimicrobial resistance genes can be ingested via raw milk consumption (Liu et al., 2020). To mitigate the risk, any milk, that enters the market is screened for antibiotic residues and will be immediately discarded, if found positive. However, in the case of direct sales of raw milk on farms, these aspects have to be kept in mind as that milk is not tested.

To counteract these health hazards, there are a variety of preventive measures, from the stable to the finished product. By law, the bacterial count of tank milk must not exceed 100,000 cfu/mL and the cell count must not exceed 400,000 cells/mL, and no traceable antimicrobial inhibitors must be present (RohmilchGütV). Otherwise, if the threshold values are exceeded, deductions will be made, and milk deliveries may be refused (RohmilchGütV).

In the processing of milk and milk products, the milk is usually thermally treated (e.g., pasteurization, ultra-high temperature), which can ensure a largely safe product for the consumer (Lucey, 2015). In general, the risk of infection with a zoonotic pathogen from processed milk in the age of pasteurization is considered low (Bradley, 2002). However, so-called certified raw milk (in German: Vorzugsmilch), i.e., packaged and merely filtered raw milk, can also be freely purchased and on-farm purchases of raw milk are also possible in Germany. When selling raw milk on-farm, the farm must comply with the standard legal hygiene requirements for dairy farms and always display a "boil before consumption" sign. In the case of certified raw milk, clear legal requirements apply (Tier-LMHV).

Despite the aforementioned precautions, minimizing risk by reducing udder diseases is paramount. Udder diseases can have an impact on food safety and hygiene in several ways.

Mastitis pathogens, resistant pathogens, or antimicrobial inhibitors can enter the food chain through milk from infected (and treated) cows. Open wounds as in udder cleft dermatitis and udder thigh dermatitis can result in contamination of the milking equipment and consequently the milk itself with blood or pus or both.

Hence, ensuring safe food and milk quality starts in the barn. Here, the animals require observation; among other things, they must not have any infectious diseases that can be transmitted to humans via milk, must generally be in good general health, and must not have any udder wounds that could lead to contamination of the milk (Regulation (EC) No 853/2004, European Commission, 2004).

In addition, there are detailed regulations to minimize the risk of contamination from milking equipment, storage facilities, etc. (Regulation (EC) No 853/2004, European Commission, 2004).

2.3. Animal welfare

"An animal is in a good state of welfare if (…) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear and distress" (World Organisation for Animal Health, OIE, 2013). Animal welfare is defined by the five freedoms. These include "the freedom from hunger and thirst, (…) from discomfort, (…) from pain, (…) injury and disease, (…) to express normal behaviour [and] (…) from fear and distress" (Farm, Animal Welfare Council, FAWC, 2009). Udder diseases, especially mastitis, have a negative impact on animal welfare. Fitzpatrick et al. (1998) found increased pain sensitivity in cows with mastitis, in both severe and moderate cases. Changes in normal behaviour may indicate pain and thus reduced welfare (Weary et al., 2006). Mastitis leads to such changes, but also to physiological abnormalities. Animals, suffering from CM, may have higher body temperature, heart as well as respiratory rates compared to healthy animals.

In terms of behaviour, cows experimentally infected with *Escherichia* (*E.*) *coli* have been found to have a reduced feeding time despite slower eating (Siivonen et al., 2011; Fogsgaard et al., 2012). Cows with mastitis lie less on the diseased side, have reduced lying times, walk more (Siivonen et al., 2011), and stand with the hocks further apart (Kemp et al., 2008). It should be noted that the increased activity deviates from classical disease findings of decreased activity in otherwise diseased animals (Dantzer, 2001). This is probably because the cows want to avoid the pain caused by pressure on the udder when lying down (Siivonen et al., 2011). There is also a decrease in rumination time and self-grooming, both of which are typical for good welfare (Fogsgaard et al., 2012). Even if there is no specific data on this, it can be assumed that other udder diseases such as UTD or UCD are also painful and will lead to a reduced well-being.

3. Mastitis

Mastitis is "an almost exclusively infectious inflammation of the mammary gland" (Wiesner and Ribbeck, 2000). Because of its consequences for individual animal welfare, its financial costs to dairy farms, as well as its potential impact on food and consumer safety, mastitis is considered the most important udder disease worldwide. Due to its importanc, a 5-point mastitis control plan was developed back in the 1960s (Neave et al., 1969; Dodd et al., 1969), which has been widely used, especially in Western countries. By following the five main points (post-milking teat disinfection, clinical mastitis treatment, dry cow treatment, culling of chronic cases, milking machine maintenance), a significant reduction of cow-associated pathogens could be achieved, with a simultaneous relative increase of environmentally associated pathogens. Due to the increase in environmental pathogens and the ongoing development in the dairy farming, the approaches to mastitis control in the modern dairy industry had to be expanded. Thus, the 5-point plan was modified and extended to a 10-point plan by the NMC, named “The recommended mastitis control plan” (current version: NMC, 2020). This includes establishing udder health goals, providing a clean and dry environment, good record keeping (e.g., of mastitis cases), and regular surveillance of udder health status.

3.1. Classification of mastitis

Mastitis can be classified in multiple ways. One option is the classification according to bacteriological-cytological aspects (normal secretion, latent infection, non-specific mastitis; DVG, 2012). Other possibilities are the classification based on the course of the infection (acute, chronic), the morphology (apostematic, toxic, ...) or based on the etiology (e.g., coli mastitis).

Frequently used by practitioners is the classification based on clinical manifestation or symptoms. This classification was also chosen in many studies (Hiitiö et al., 2017; Detilleux, 2018) and also in our study. Basically, two forms can be differentiated: clinical mastitis and subclinical mastitis.

Clinical mastitis is defined by at least visual changes in the milk (International Dairy Federation, IDF, 2011). This includes flakes, blood, pus, or up to such strong secretion changes that such is no longer recognizable as milk. The SCC usually exceeds 200,000 cells/mL (NMC, 2001). In addition, there may be clinical findings in the udder (induration, redness, pain) or in the animal (disturbed general condition, fever, inappetence, recumbency).

Unlike CM, SUBM cannot be diagnosed by sensory examinations alone and requires diagnostic tools (such as the California mastitis test (CMT); IDF, 2011.) Subclinical mastitis is characterized by an increase in SCC (≥ 100,000 cells/mL; DVG, 2012), often accompanied by a decrease in milk production (Halasa et al., 2009) or identification of udder pathogens, or all three.

4. Mastitis pathogens

In addition to bacterial mastitis, mycoses (e.g., yeasts, especially *Candida* spp,; Wawron et al., 2010) or algae (*Prothoteca* spp.; Jagielski et al., 2019) can also cause mastitis. Whether viruses directly or indirectly are associated with mastitis has been debated, e.g., through their immunosuppressive effect (Wellenberg et al., 2002).

Bacterial mastitis pathogens can be subdivided based on their pathogenicity. Pathogens with higher pathogenicity are classified as major pathogens. However, pathogens that are considered to play a minor role are classified as minor pathogens (Godden et al., 2003). A selection of mastitis pathogens will be briefly summarized below.

4.1. Major pathogens

Major pathogens typically lead to higher cellular response in the udder, milk yield decreases or clinical courses, or all three (Griffin et al., 1977). Among major pathogens, cow-associated pathogens are again differentiated from environment-associated pathogens, although the definite initial assignment for one or the other pathogen is not as clear-cut as long assumed (Bradley, 2002). The assignment should no longer be made at the species level but at the strain level (Zadoks et al., 2011). Strain heterogeneity within a herd is considered evidence of environmental mastitis. In contrast, strain homogeneity is not considered definitive evidence for cow-associated pathogens, as it does not have to be clearly a contagious infection but can also be due to a point infection in the environment (Klaas and Zadoks, 2017).

In the following, the description of the individual pathogens will also include the assignment in more detail.

4.1.1. Cow-associated major pathogens

Transmission of cow-associated pathogens occurs from cow to cow. Poor milking hygiene, especially transmission via milkers' hands and milking equipment, poses a major risk (Fox and Gay, 1993).

The pathogens considered to be classical contagious are *S. aureus* and *Streptococcus* (*Str.*) *agalactiae*, but also *Str. dysgalactiae* and *Str. canis*. However, there are some studies that also attribute an environmentally associated route of transmission to *S. aureus* and *Str. dysgalactiae* or assign them to both categories (Wente and Krömker, 2020).

4.1.1.1. *Staphylococcus* (*S.*) *aureus*

S. aureus are gram-positive cocci from the genus *Staphylococcus* spp. (Winter, 2009a) and can be found as a commensal on skin and mucosa but are also frequently detected in skin lesions or decubitus (Capurro et al., 2010; Cicconi-Hogan et al., 2013). Infection of the udder with *S. aureus* occurs predominantly subclinically and is associated with high SCC (Petersson-Wolfe et al., 2010). Clinical courses are also possible (Keane et al., 2013). In chronic courses, granuloma and nodule formation is likely that will impair treatments success (Winter, 2009a). In principle, *S. aureus* is classified as a contagious pathogen (Fox and Gay, 1993). However, studies showed that *S. aureus* may also exhibit characteristics of an environmental pathogen in terms of epidemiology and response to management changes (Zadoks et al., 2002; Sommerhäuser et al., 2003).

Although the 5-point mastitis control plan primarily targets contagious pathogens like *S. aureus*, it is not as facile to control as, for example, *Str. agalactiae* (Zadoks and Fitzpatrick, 2009). In addition to the common routes of transmission for cow-associated pathogens, transmission via flies has also been reported (Capurro et al., 2010; Anderson et al., 2012).

Subclinically, persistently infected cows are considered a substantial reservoir. Therefore, in herds with *S. aureus*, particular emphasis should be placed on good milking hygiene, infected cows should be held separated, milked last, or culled in persistent cases (Petersson-Wolfe et al., 2010).

4.1.1.2. *Streptococcus* (*Str.*) *agalactiae*

Str. agalactiae belong to the aesculin-negative streptococci and are also gram-positive cocci. *Str. agalactiae* is also considered a primarily contagious pathogen, but again, studies show a more complex epidemiology. In his study, Jørgensen et al. (2016) also detected *Str. agalactiae* in faeces, alleys and water troughs, for example, and hence did not exclude an oro-faecal infection.

For a long time, it was erroneously believed that *Str. agalactiae* was an obligate intramammary pathogen (McDonald, 1984). However, in addition to the detection in the environment (Jørgensen et al., 2016), the detection of *Str. agalactiae* in humans (Zhao et al., 2008) and other animals, e.g., dogs and cats (Yildirim et al., 2002), disproves this assumption. *Str. agalactiae* mastitis mostly occurs subclinically, yet it can also manifest chronically, sometimes ending into quarter atrophy (Winter, 2009a). Infection with *Str. agalactiae* is associated with high quarter-level SCC (Djabri et al., 2002). Since the introduction of the 5-point mastitis control plan, the prevalence of *S. agalactiae* has decreased noticeably. In addition to standard *S. agalactiae* prevention programs, antibiotic therapy with penicillin generally responds well (Huber-Schlenstedt et al., 2017). However, therapy failures need to be culled to eradicate reservoirs.

4.1.1.3. ***Streptococcus* (*Str.*) *dysgalactiae***

Str. dysgalactiae also belong to the gram-positive cocci of the genus *Streptococcus* spp. (Winter, 2009a). Like *S. aureus* and *Str. agalactiae*, *Str. dysgalactiae* was originally classified as a cow-associated pathogen (Fox and Gay, 1993). This classification also is no longer clear-cut. *Str. dysgalactiae* can be assigned to both categories, cow- and environment-associated. Wente and Krömker (2020) could identify up to seven strains of *Str. dysgalactiae* in one herd. *Str. dysgalactiae* occurs as a pathogen in both, SUBM and CM, with subclinical courses predominating (Winter, 2009a). Infection with *Str. dysgalactiae* results in high shedding rates of this pathogen (Hamel et al., 2021) and milk losses (Heikkilä et al., 2018).

4.1.2. Environment-associated major pathogens

As mentioned before, the 5-point mastitis control plan focused on contagious pathogens, which led to a relative increase in environmental pathogens such as *Str. uberis* and *E. coli* (Zadoks and Fitzpatrick, 2009). While contagious pathogen prevalence mainly depends on milking hygiene, environmental pathogen prevalence depends on both hygiene in the barn, e.g., clean cubicles (Schukken et al., 1990), as well as milking hygiene, e.g., pre-milking teat disinfection (Oliver et al., 1993; Bradley et al., 2018).

4.1.2.1. ***Streptococcus* (*Str.*) *uberis***

Str. uberis is the most important representative of the aesculin-positive streptococci. It predominantly shows characteristics of an environmentally associated pathogen (McDougall et al., 2004), but cow-to-cow transmission has also been described more frequently (Zadoks et al., 2003; Davies et al., 2016; Leelahapongsathon et al., 2020).

Str. uberis is an ubiquitous pathogen found predominantly in the environment of cattle. It colonizes various body sites of cows, including the digestive tract (Kruze and Bramley, 1982), and is thus distributed into the environment through the faeces (Zadoks et al., 2005; Sherwin and Breen, 2022). In the barn, *Str. uberis* is found throughout the cow's environment, especially in the waiting area in front of the milking parlor and in the bedding (Wente et al., 2019). *Str. uberis* can cause SUBM and chronic mastitis but counts among the most important pathogens in CM (McDougall et al., 2007; Winter, 2009a; Huber-Schlenstedt et al., 2017). It has also been isolated in cows with metritis (Wagener et al., 2014), whereas in other animals *Str. uberis* infection is not known.

4.1.2.2. ***Escherichia* (*E.*) *coli***

E. coli is a gram-negative rod, that can be found ubiquitously in the stable and is physiologically shed with feces (Burvenich et al., 2003). *E. coli* mastitis mostly affects high-producing cows (Burvenich et al., 2003).

After entering the udder (typically via the streak canal) the multiplication, death, and lysis of *E. coli* releases, among other things, an endotoxin of the cell wall, the lipopolysaccharide (LPS). The LPS is an initiator of the immune response and activates the signalling cascade and cytokine release (Burvenich et al., 2003). Polymorphonuclear neutrophils (PMNs) are activated to fight the pathogens (Burvenich et al., 1999). A suppressed immune system, especially in peripartum cows, can lead to a failure of immunoregulatory mechanisms and to a delayed reaction (Waldmüller, 2012). The resulting initially uncontrolled multiplication of *E. coli* and associated high toxin concentration leads to a delayed, but excessive immune response with severe clinical symptoms and up to multi-organ failure (Burvenich et al., 2003). This makes *E. coli* the most common cause in fatal mastitis (Hazlett et al., 1984; Menzies et al., 1995). However, *E. coli* mastitis can also have a moderate to mild course, especially in cows in mid-lactation (Waldmüller, 2012).

4.2. Minor pathogens

Minor pathogens are mostly colonizers of the teat canal or commensals of the teat skin (Devriese and Keyser, 1980). Opportunistically, they infect the udder, but only in a few cases lead to an increase in the SCC or even to a clinical manifestation (Griffin et al., 1977).

4.2.1. Coagulase-negative Staphylococci (CNS)

The coagulase-negative staphylococci comprise a large group of staphylococci (Vanderhaeghen et al., 2015). The main CNS pathogens identified are *S. chromogens*, *S. simulans.*, *S. xylosus*, *S. epidermidis*, *S. warneri* and *S. haemolyticius* (Sampimon et al., 2009; Supré et al., 2011).

Although CNS are usually counted as a homogeneous group, species-specific differences are known (Supré et al., 2011) and CNS are among the most common pathogens isolated from milk samples in many countries (Pitkäla et al., 2004; Tenhagen et al., 2006; Poutrel et al., 2018).

Mastitis with CNS tends to remain subclinical (Taponen and Pyörälä, 2009), with persistent infections being possible (Chaffer et al., 1999). They are often isolated in clinically healthy primiparous cows (Tenhagen et al., 2006).

Infections with CNS usually result in only a slight increase in SCC (Djabri et al., 2002). It is debated whether CNS protects udders from infections with other pathogens, leading to different conclusions. On the one hand, studies showed that IMI with CNS was associated with an increased risk of IMI with major pathogens. For example, one study showed, the risk of infection with *S. aureus* or *Str. uberis* post calving was increased in precalving IMI with CNS (Parker et al., 2007). In contrast, a study by dos Santos Nascimento et al. (2005) found an inhibitory effect of CNS strains on *Str. agalactiae* strains. Another study attributed neither a protective nor a predisposing effect to CNS regarding IMI with *S. aureus* or *Str. uberis* (Zadoks et al., 2001).

4.2.2. *Corynebacterium* spp.

Corynebacterium spp. is a pleomorphic, gram-positive bacterium. The main representative of the *Corynebacterium* spp. is *Corynebacterium* (*C.*) *bovis*. As with CNS, infection with *C. bovis* results in only slight increases in cell number. In a meta-analysis, Djabri et al. (2002) found a geometric mean SCC of 105,000 cells/mL in IMI with *C. bovis*. While *Corynebacterium* spp. has been identified as a risk factor for clinical mastitis just prior to the dry period, it has conversely been attributed a protective effect when isolated in the late dry period or post calving (Green et al., 2002).

5. Other udder diseases

The focus of udder health is often set on mastitis, but other udder diseases also play an important role in udder health, especially regarding animal welfare and food safety. To the best of our knowledge, there are no recent data on the prevalence of udder diseases such as teat warts, udder edema, udder thigh dermatitis, udder cleft dermatitis, or other in Bavaria, Germany. The diseases of the udder (addressed in the first publication), namely teat warts, udder edema, udder thigh dermatitis, and udder cleft dermatitis, will be reviewed in the following.

5.1. Teat warts

Teat warts are caused by the bovine papillomavirus (BPV). The warts appear as thick nodules in the skin. They do not show any redness nor are they painful. They can look rice grain-like, have a thick base or form secondary and even tertiary papilloma on top of the primary tumor similar to a benign tumor (Kirk and Sischo, 2003). Usually the warts heal spontaneously, but some persist. Persistency occurs mainly in immunocompromised animals (Nasir and Campo, 2008). Due to teat warts, problems during milking, due to difficulties in attachment of the milking clusters, and suckling problems for calves can occur. If the prominent parts of teat warts are torn off or injured, the wound may become infected or lead to mastitis (depending on the localisation) or even narrowing of the teat canal (Kirk and Sischo, 2003; Schukken et al., 2003; Kale et al., 2018).

There is scarce data on the occurrence of teat warts, none are known for Germany. Nouh et al. (2014) reported an incidence of 1.7% for teat warts in an Egyptian retrospective study. Nooruddin et al. (1997) found a prevalence of 16% in Bangladesh. In a Dutch study, the proportion of affected teats with warts decreased from 22 to 14% after changing from conventional milking to automatic milking systems on 15 farms (Neijenhuis et al., 2004).

Various treatment approaches can be found in the literature, including surgical removal (Stöber, 2006), paramunity inducer (Turk et al., 2005), or autogenous vaccines (Kale et al., 2018). There are only few case reports about the efficacy of autogenous vaccines. Mostly they were used in combination with other therapies and showed a successful reduction and prevention of recurrence of warts (Turk et al., 2005; Ranjan et al., 2013; Mayilkumar et al., 2014; Kale et al., 2018).

It is possible (in Germany according to the Regulation on Animal Vaccines, TierImpfStV) to have autogenous, i.e., farm-specific vaccines produced in case of herd problems (Stöber, 2006). To prevent infections, good (milking) hygiene and purchase controls are recommended (Stöber, 2006). No extensive studies on teat wart prevalence or risk factors have been done so far in Germany.

5.2. Udder edema (UE)

Udder edema is the pathological accumulation of fluids in udder tissue due to inflammation or reabsorption and filtration disorders of the local vascular system (Wiesner and Ribbeck, 2000). In a study by Kojouri et al. (2015) low levels of total protein and lipid markers as well as lipoproteins in the blood of primiparous cows with UE were noticed.

It is a common condition and affects about 70% of cows around calving (Morrison et al., 2018). Various risk factors are described, with partly different results. While Melendez et al. (2006) described an increased occurrence of UE in cows giving birth to heavy calves, Malven et al. (2006) saw higher rates of it in cows with light calves. Season also had an influence on the occurrence of UE in Melendez et al.'s (2006) study, but not in Dentine and McDaniel's study (1983). Feeding high-energy diets to dry cows increased the risk of UE (Johnson and Otterby, 1981) as well as feeding highly fermentable diet in the periparturient time (Radostits et al., 2000). First-time heifers are particularly affected (Dentine and McDaniel, 1983). Offspring of high-yielding cows have an increased risk of UE (Shanks et al., 1978). The associated swelling leaves the udder more exposed to kicking injuries and, if chronic, can cause permanent damage to the suspensory apparatus (Loppnow, 1959). The friction of the swollen udder against the inner thigh poses be a risk factor for udder thigh dermatitis (Sigmund et al., 1980; Roy et al., 2012). For mild cases, UE usually resolves on its own within a few days, massages and exercise can promote regression. In more severe cases, diuretics and antiphlogistics can be used for therapy (Winter, 2009b).

5.3. Udder thigh dermatitis (UTD, Intertrigo)

Similar to UE, there are not many studies on prevalence of or risk factors for UTD, also often called intertrigo. Intertrigo is a dermatitis caused by friction between two adjacent surfaces (Wiesner and Ribbeck, 2000), in the case of UTD, the friction between the udder and the inner thigh. After the first stage with erythema, the affected area begins to swell. Then the hair becomes matted, the area becomes soggy and there may occur systemic signs such as fever and recumbency. Bacteria invade the lesions and secondary infections can develop. The spot may demark, and cows may also become lame and lie down more (Roy et al., 2012). Once the edema subsides, healing, granulation and re-epithelialisation ensue due to a decrease in friction (Sigmund et al., 1980). The most commonly identified pathogen in UTD is *Fusobacterium necrophorum*, an anaerobe, but also *Corynebacterium spp*. (Roy et al., 2012). Sigmund et al. (1980) on the other hand, examined only aerobic pathogens and found *Corynebacterium spp.* to be the most common pathogen, but also gram-negative pathogens such as *E. coli* and *Proteus vulgaris* were present. Heifers are more frequently affected than cows. In a German study, an incidence was at 1% for heifers and 0.06% for multiparous cows (Sigmund et al., 1980). In contrast, in France the incidence was higher: 23% and 1.2%, respectively (Roy et al., 2012). Udder thigh dermatitis is associated with UE, which causes friction between the inner thighs and the udder due to swelling. In a study by Roy et al. (2012), 98% of the cows with UTD also showed UE.

The first step in treating UTD is to control the usually present UE (Roy et al., 2012). The affected areas should be shaved out and wound debridement should be performed (Winter, 2009c). Furthermore, lavage with disinfectant agents is recommended (Sigmund et al., 1980). Systemic antibiotics may only be necessary for large deep wounds (Winter, 2009c).

5.4. Udder cleft dermatitis (UCD)

With UCD, skin lesions are found on the udder, usually between the two forequarters or at the cranial transition between the udder and the abdominal wall (Warnick et al., 2002). A distinction is made between the mild and the severe course, which can lead to open skin areas and even deep ulcers and necrosis (Ekman et al., 2018). In severe cases, it can result in death either from embolic pneumonia (Millar et al., 2017) or when penetrating the mammary vein (Bouma et al., 2016).

No single infectious cause has been confirmed. The diversity of the bacteria is lower compared to the healthy skin areas, a dysbiosis occurs (Sorge et al., 2019; Ekman, 2020). While staphylococci predominate in the mild form, the frequency of anaerobes such as *Fusobacteria* spp. and *Trueperella* spp. increases in the severe form but no specific pathogen could be attributed to the development of UCD (Ekman 2020). Sorge et al. (2019) could not detect any viral, fungal or mange components in their samples when investigating UCD histologically. On the other hand, the sores were associated with the multitude of *Fusobacterium*, *Porphyromonas* spp., *Helococcus*, and other bacteria (Sorge et al., 2019) - the disease is likely multifactorial.

In the Netherlands, 5.2% of cows were affected with UCD, and the within-herd prevalence varied between 0 and 15% (Olde Riekerink et al., 2014), whereas in Sweden 18% of cows were affected and the within-herd cow prevalence ranged from 0 to 39% (Persson Waller et al., 2014). In another Swedish study, 28% of cows were found positive for UCD, with the mild form of UCD being found more frequently (19% of cows) than the severe form (9% cows, Ekman et al., 2018). The within-herd prevalence there ranged between 0 and 62%. There are currently no data on the prevalence of UCD in Germany, or specifically Bavaria.

Unlike udder edema and UTD, UCD is more common in older cows than in primiparous cows (Warnick et al., 2002; Persson Waller et al., 2014; Bouma et al., 2016; Ekman et al., 2018). The disease also seems to occur more frequently at high milk production levels (Olde Riekerink et al., 2014; Persson Waller et al., 2014; Ekman et al., 2018). Additionally, udder conformation plays a role. Deep udders in relation to the hock and large forequarters seem to be more susceptible to UCD (Olde Riekerink et al., 2014).

Mastitis is thought to be associated with UCD. In Persson Waller et al.'s study, the risk of veterinary-treated clinical mastitis was higher for cows with UCD than for cows without UCD (Persson Waller et al., 2014). They concluded that UCD increases the risk of veterinary-treated clinical mastitis because skin lesions, such as hock lesions, provide a reservoir for *S. aureus* (Capurro et al., 2010).

As with UTD, the wound should first be cleaned and debrided (Ekman, 2020). Lammers et al. (2017) attributed a healing-promoting effect to a chelated copper and zinc spray, but Ekman (2020) saw no general effect on wound healing for this. Furthermore, Ekman (2020) recommended adapting the topical application of products to the condition of the wound, such as medical honey for secondary bacterial infections. In a study by van Werven et al. (2018), enzyme alginogel was shown to be effective in treating severe UCD.

III. PUBLICATIONS

1. Publication I

Kumulative Promotionsleistung: Publikation

Apparent prevalence and risk factors for udder skin diseases and udder edema in Bavarian dairy herds

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Apparent prevalence and risk factors for udder skin diseases and udder edema in Bavarian dairy herds

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ABSTRACT

The objective of this cross-sectional study was to assess the prevalence and risk factors for teat warts, udder edema, udder thigh dermatitis, and udder cleft dermatitis on Bavarian dairy farms. Udder health and hygiene scores of lactating cows were recorded on 152 farms in Bavaria, Germany. Management practices (e.g., housing, milking systems, and feeding regimens) were assessed with a comprehensive questionnaire. Adjusted prevalence estimates were determined using regression analysis with herd as the random effect. Mann-Whitney U or Fisher's exact on herd level and regression analyses on cow level were performed to determine risk factors. Of the 6,208 cows examined, 4.0% had teat warts, 1.1% udder edema, 0.2% udder thigh dermatitis, and 0.3% udder cleft dermatitis. The apparent median within-herd prevalence was less than 4% for all 4 diseases. Herd-level factors that were associated with the presence of teat warts on a farm were the proportion of cows with poor teat ends as well as conventional milking systems compared with milking robots. At a cow level, teat warts were associated with high somatic cell counts. Herds with poor depth (<5 cm) of bedding material and cows with days in milk less than 60 d had increased odds for udder edema. First-lactating cows had higher odds for udder thigh dermatitis. Freestall housing and comfort rubber mats were identified as risk factors for udder cleft dermatitis on a herd level. In conclusion, although most nonmastitis udder diseases were rarely observed in this study, some herd management practices and cow factors were associated with their presence on a farm or cow level. Future studies are needed to further investigate risk factors for each disease in more detail.

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Key words: teat wart, udder edema, udder thigh dermatitis, udder cleft dermatitis, management

INTRODUCTION

When it comes to bovine udder diseases, mastitis comes first and foremost to mind. However, other udder diseases also have to be considered. These include udder edema (**UE**) as well as the skin diseases: teat warts, udder thigh dermatitis (**UTD**), and udder cleft dermatitis (**UCD**). They can be relevant for animal welfare (Okkema and Grandin, 2021) and food safety (Regulation 853/2004, European Commission, 2004).

Though there are many studies on the association of management practices and risk factors for mastitis (Santman-Berends et al., 2016; Hiitiö et al., 2017; Duse et al., 2021), there is scarce information on the prevalence or risk factors of the diseases just mentioned. Therefore, we will discuss the 4 diseases and some of their known risk factors.

Udder and teat warts are predominantly found on teat skin (teat warts) and are small skin nodules caused by *Papillomavirus bovis* (Jarrett et al., 1984; Rai et al., 2011; Kumar et al., 2013). They can grow to the size of a grain of rice or take on secondary and tertiary formations (Kirk and Sischo, 2003). In some cases, they affect the fit of liners and cause difficulties during milking. In severe cases, they may even tear off, the sites may become infected, and, depending on the localization of the lesion, they may even lead to intramammary infections (Kirk and Sischo, 2003; Campo, 2006). To the best of our knowledge, there are no formal studies focusing on risk factors for teat warts. One study (Neijenhuis et al., 2004) reported that changing from conventional to automatic milking systems (**AMS**) reduced the occurrence of teat warts in 15 Dutch herds, and a Japanese study speculated that fly infestation of pastures may be a risk factor for the development of teat warts (Maeda et al., 2007).

Udder edema is the pathological accumulation of fluids in the udder tissue that occurs in about 70% of cows around calving (Morrison et al., 2018). The swelling associated with UE increases the risk of teat and udder injury (Dentine and McDaniel, 1983), as well as UTD (Roy et al., 2012). Furthermore, chronic UE can lead to damage of the suspensory apparatus of the udder (Loppnow, 1959). Morrison et al. (2018) reported an increase in the incidence of clinical mastitis with UE. It also causes difficulties in attaching the milking cluster. Feeding practices, such as the feeding of a highly fermentable diet in the periparturient time (Radostits et al., 2000) or the feeding of large amounts of salt (Lema et al., 1992), can influence the severity of UE.

Udder thigh dermatitis usually develops as a result of UE, due to friction between the udder and the inner thigh, especially in primiparous cows. In more severe cases, the resulting skin lesions can become secondarily infected with bacteria and lead to fever and lameness. Cows in tiestalls have an increased risk of developing UTD due to the reduced opportunity for locomotion compared with cows in loose stalls and straw yards (Roy et al., 2012).

Another type of dermatitis is UCD. Skin lesions form mostly on the cranial udder and in multiparous cows (Persson Waller et al., 2014). Severe cases have been associated with embolic pneumonia (Millar et al., 2017) or even the death of the animal when lesions penetrated the milk vein (Bouma et al., 2016). As in UTD, no single causative infectious agent was identified (Sorge et al., 2019).

The open wounds associated with UTD and UCD may contaminate the milk with blood or pus at harvest and may increase the risk of intramammary infections. According to a recent Swedish study (Ekman et al., 2018), cows housed on comfort mattresses had an increased risk compared with cows housed on other cubicle bedding.

The authors are not aware of any prevalence data for these diseases in Germany. Therefore, the objectives of this study were (1) to determine the prevalence of teat warts, UE, UTD, and UCD on Bavarian dairy farms and (2) to investigate the associations of cow factors and management practices with these diseases.

MATERIALS AND METHODS

Herd Selection

This cross-sectional study was conducted between September 2017 and June 2018 in Bavaria, southern Germany. A list of all Bavarian dairy farms (n = 28,884) was used as the basis for enrollment. The list did not include herd size but only daily milk shipped. The authors assumed that herds producing less than 200 kg milk per day (n = 4,873) had fewer than 10 lactating dairy cows. These herds were excluded. The list of remaining herds (n = 24,011) was quartered based on the amount of daily shipped milk. After randomly assigning a number to each farm, the 4 lists were sorted by random number. The first 200 farms per stratum were selected as the recruitment list, as we were expecting a response rate of 30% and 200 herds was deemed sufficient to recruit 40 herds per stratum. The 4 lists were then split by area code and distributed respectively among the 10 branches of the Bavarian Animal Health Services, covering all Bavarian regions. The herds were then recruited by phone by Bavarian Animal Health Services technicians along the provided lists for each branch. Animal and human ethical guidelines were followed during this study. The sample collection did not require ethical approval under the German animal welfare law.

This study was part of a larger study that primarily focused on the prevalence of mastitis pathogens and management practices in Bavaria. For the main study, up to 100 lactating cows were examined per herd. The 100 cows to be examined in herds with more than 100 cows (n = 3) were randomly selected in advance from a list by the technicians. Because we expected the prevalence of the described diseases to be very low and the sample size was driven by funding restraints for the main study, we did not perform any additional sample size calculation for any of these diseases.

Herd Visit

The farms were visited once by qualified Bavarian Animal Health Services technicians (n = 20) in teams of 2. The detection of udder diseases is part of the daily job of the technicians, and they receive regular training. In addition, they were briefed again before the study with in-class training and information sheets. However, no formal assessment of interobserver agreement was conducted. During the visit, the technicians visually and palpatorically assessed all udders for the aforementioned diseases. A cow was considered positive for teat warts if at least one teat wart was detected, from a raised nodule to larger proliferations on a teat. Udder edema was defined as at least a slight degree of edema, pasty swelling of the udder (i.e., a fingerprint can be seen for a brief moment, or the floor of the udder was too firm for any imprint) without any palpatory or visual signs of inflammation (heat, redness, or pain). A cow was found UCD positive when skin lesions (in the form of crusts, wounds, thickened skin, or exudations of various stages) were observed adjacent to the cranial udder or in the skin fold between the udder halves.

Udder thigh dermatitis was defined as skin lesions between the udder and the inner thigh. The skin lesions could range from superficial skin lesions to deep open wounds, including exudation and smell (Sigmund et al., 1980).

The technicians also assessed teat end condition on a cow level (score 1 to 4, highest score recorded per cow; NMC, 2007), hock lesions (score 1 to 3; NMPF, 2013), udder hygiene (score 1 to 4; Schreiner and Ruegg, 2003), and leg hygiene (score 1 to 4; NMPF, 2013). In addition, a quarter-level California Mastitis Test was performed, and quarter milking samples were taken from all study cows and analyzed for mastitis pathogens (results not shown). Furthermore, a checklist (available upon request) was completed during the farm visit regarding the herd's management practices (e.g., milking routine, dry-off procedures, nutrition, and housing).

Where available, the farmers provided monthly milk test results (DHIA report), the data set of the AMS, or both at the time of visiting and sampling. The monthly DHIA report included the results of the latest test day, providing information on cow data such as lactation number, DIM, breed, and milk parameters (milk protein, fat and urea concentration, test day milk yield, and SCC).

Statistical Analyses

Data were analyzed using SAS 9.4 (SAS Institute Inc.). Alpha was set at 0.05.

First, the data were checked for completeness and plausibility (i.e., unlikely values). Descriptive statistics were performed with PROC MEANS for continuous data and PROC FREQ for categorical data. Based on the call lists (i.e., herd size), the herds were placed into 4 groups, with group 1 having the smallest and group 4 having the largest herds.

Cow-level prevalence analysis was performed using PROC GLMMX with herd as a random effect to account for a farm effect.

On-farm cow data were merged with the monthly DHIA test data, where available. For each disease, variables of the questionnaire, on-farm observations, and the DHIA data were selected to identify risk factors for herd prevalence or disease status of the cow. Possible predictors were chosen based on biologically plausible associations, including known risk factors, as well as other potential associations.

Because of the overall sparsity of cases for the various diseases, the herd-level analysis only allowed for a comparison of individual risk factors between herds with or without disease. Potential risk factors were screened using Mann-Whitney U for continuous data and Fisher's exact test for categorical data. For these analyses, the observed teat end scores greater than or equal to 3 were summarized per herd as 0%, $> 0\%$ to 10%, and $>10\%$ of cows per herd. Multivariate analyses of potential herd-level risk factors did not converge despite, for example, adjustments in maxiter function.

Cow-level risk factor analysis included only cows from herds in which a particular disease occurred. Analyses were performed per disease to identify potential cow-associated risk factors (predictors), such as lactation number (1, ≥ 2), breed (Simmental, Brown Swiss, other), or milk yield (kg). The variable DIM was dichotomized as ≤ 60 DIM and >60 DIM. Cow SCC was divided into $\leq 100{,}000$ cells/mL and $>100{,}000$ cells/mL according to Harmon (1994). Udder and leg hygiene scores were categorized into clean (scores 1 and 2) and dirty (scores 3 and 4). Cow milk yield, milk protein concentration (%), and milk urea concentration (mg/kg) were categorized based on percentiles. An overview of all cow-level factors screened for each disease can be found in Table 3.

Collinearity of predictors was assessed using Spearman rank correlations for continuous or kappa for categorical predictors (PROC FREQ AGREE). If either the correlation coefficient or kappa was greater than 0.6, the variable with less value was excluded. Then identified predictors were included individually in a mixed logistic regression (PROC GLIMMIX) that also included herd as a random effect. Variables with $P < 0.25$ were kept for further analysis in multivariable logistic regression models. Last, a multivariable logistic regression analysis (PROC GLIMMIX) with herd as a random effect was performed using a "quasi-backward" selection—that is, factors (including their interactions) were individually removed or placed back into the model at a later point to assess their effect (alone or as a biologically plausible interaction) on other variables. If a change in coefficient greater than or equal to 20% occurred in other variables, that variable remained in the model as a potential confounder. Otherwise, only variables with $P < 0.05$ remained in the final model. Because only models with one variable remained, no meaningful model comparison (e.g., Akaike information criterion comparison) could be performed.

RESULTS

Herd Description

Based on the lists for each branch, 339 farms were contacted and 156 accepted (response rate = 46%). The larger the farms, the more likely they were to participate. The response rate was 35% for group 1, 41% for group 2, 49% for group 3, and 57% for group 4. Of these, 4 farms backed out before the visit, so that only 152 herds were visited in total. The farms were visited between September 2017 and June 2018, and a total of

Table 1. Description of the Bavarian study herds sorted by group[1]

Variable	Group 1 (n = 38)	Group 2 (n = 37)	Group 3 (n = 39)	Group 4 (n = 38)	Overall (n = 152)	*P*-value[2]
Herd size, mean ± SD	20 ± 4[d]	31 ± 4[c]	51 ± 6[b]	86 ± 42[a]	48 ± 33	<0.01
Herd size, minimum–maximum	12–26	27–40	41–61	62–327	12–327	
Rolling herd average[3,4] (kg; IQR)	7,199[bc] (6,299–8,095)	7,175[c] (6,484–7,948)	7,901[b] (7,188–8,505)	8,708[a] (8,279–9,116)	7,906 (6,884–8,626)	<0.01
Bulk tank SCC[3,5] (×1,000 cells/mL; IQR)	146 (105–200)	150 (111–189)	138 (102–179)	150 (112–200)	147 (107–190)	0.83
Bulk tank bacterial count[3,5] (×1,000 cfu/mL; IQR)	18 (12–33)	16 (8–24)	12 (8–19)	13 (8–24)	14 (8–23)	0.10
Housing type (% herds)						
Tiestall	86.8[a]	62.2[b]	15.4[c]	2.6[c]	41.5	<0.01
Freestall	13.2[c]	37.8[b]	84.6[a]	97.4[a]	58.5	<0.01
Operating structure (% herds)						
Conventional	89.5	81.1	87.2	94.7	88.2	0.33
Organic	10.5	18.9	12.8	5.3	11.8	0.33
Milking system						
Conventional	100.0[a]	97.3[a]	94.9[a]	71.1[b]	90.8	<0.01
Automatic milking system	0.0[b]	2.7[b]	5.1[b]	28.9[a]	9.2	<0.01
DHIA member (% herds)	71.1[b]	89.2[b]	94.9[a]	89.5[ab]	86.2	0.02
Predominant breed (% herds)						
Simmental	78.9	70.3	82.0	71.0	75.6	0.56
Brown Swiss	13.2	18.9	15.4	5.3	13.1	0.32
Others	7.9[ab]	10.8[ab]	2.6[b]	23.7[a]	11.3	0.03

[a–d]Different superscripts indicate significant differences ($P < 0.05$) between the means of the different groups (from the largest to the smallest mean).

[1]A list of all Bavarian herds (excluding herds with <200 kg of milk shipped per day) was split into quartiles based on milk shipped daily (group). IQR = interquartile ratio; DHIA = regional DHIA, Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V. (LKV).

[2]Overall group-level comparison.

[3]Median and 25th to 75th percentile (IQR) reported, the latter in parentheses.

[4]Calculated by dividing the herd performance over the last 365 d by the average number of animals tested per day.

[5]Geometric mean of the preceding 3 mo at the time of the visit.

6,208 cows were evaluated on farm. Table 1 gives an overview of the characteristics of participating herds. Seven farms had more than 90 cows, including 2 with more than 100 cows (n = 111 and n = 121) and 1 with more than 300 cows (n = 327).

Although the majority of cows (5,616/6,208 cows) were from farms with monthly milk testing, only 4,719 cows could be matched to monthly milk recording data.

Apparent Prevalence of Udder Diseases

For the purpose of this article, apparent prevalence will simply be called prevalence.

Teat warts were observed on 55.9% of farms in 4.0% of all cows (95% CI: 3.0% to 5.3%). Among positive herds (n = 85), teat warts were recorded on 432 of the 3,277 cows.

Cows in group 4 were less likely to be diagnosed with warts (2.0%, 95% CI: 1.0% to 3.8%) than those of group 1 (7.0%, 95% CI: 4.6% to 1.05%, $P < 0.01$) and group 2 (6.5%, 95% CI: 3.9% to 10.9%, $P < 0.01$). The overall median intraherd prevalence ranged from 0.0% to 60.0% (median: 3.3%). Among herds with positive animals, the intraherd prevalence ranged from 1.5% to 60.0% (median: 9.6%).

Udder edema was observed in 1.1% of the cows (95% CI: 0.8% to 1.4%) on 29.6% of the farms. Among positive herds (n = 45), UE occurred in 79 of the 1,906 cows. The proportion of UE-positive cows did not differ between groups ($P = 0.27$). The within-herd prevalence ranged from 0.0% to 14.0% (median: 0.0%). Within positive herds, the median within-herd prevalence for UE ranged from 1.5% to 14.0% (median: 4.0%).

Udder thigh dermatitis was observed in 0.2% of all cows (95% CI: 0.1% to 0.4%) in 8.6% of all farms. Of the 666 cows in UTD-positive herds (n = 13), 15 cows were found positive for UTD. No difference in the proportion of UTD-positive cows among the 4 groups was found. The within-herd prevalence ranged from 0.0% to 13.0% (median: 0.0%). Within UTD-positive herds, the median within-herd prevalence ranged from 1.2% to 13.0% (median: 1.9%).

Udder cleft dermatitis was observed in 0.3% of cows (95% CI: 0.2% to 0.5%) in 10.5% of all herds. Among all herds positive for UCD (n = 16), the disease was found in 28 of the 914 cows. The proportion of UCD-positive

Table 2. Association between herd factors and presence of teat warts, udder edema, and udder cleft dermatitis on herd level (present or absent) based on Fisher's exact test ($P < 0.05$)[1]

Disease	Variable	n (+)	n (−)	OR	95% CI	*P*-value
Teat warts		85	67			
	Teat end condition score (≥3), % cows per herd[2]					
	>0–10	26	20	1.79	0.84–3.82	0.18
	>10	25	41	3.88	1.55–9.73	<0.01
	0	33	41	Referent		
	>10			2.16	0.80–5.87	0.15
	>0–10			Referent		
	Milking system					
	Conventional milking	82	57	4.80	1.26–18.20	0.02
	Automated milking	3	10	Referent		
Udder edema		45	107			
	Depth of bedding material (dry cows)[2]					
	>5 cm	4	33	0.22	0.07–0.66	<0.01
	≤5 cm	40	72	Referent		
Udder cleft dermatitis		16	136			
	Housing type					
	Freestall	15	74	12.57	1.61–97.84	<0.01
	Tiestall	1	62	Referent		
	Rubber mats—dry cow housing[2]					
	Plain rubber mats	4	54	1.09	0.26–4.59	>0.99
	Comfort rubber mats	7	15	6.88	1.78–26.63	0.01
	Other[3]	4	59	Referent		
	Comfort rubber mats			6.30	1.63–24.43	0.01
	Plain rubber mats			Referent		

[1]n (+) = number of herds with each condition present; n (−) = number of herds with each condition absent; OR = odds ratio.

[2]Information not available for all herds.

[3]Bedding surface or materials other than mats.

cows did not differ among the 4 groups ($P = 0.39$). The median within-herd prevalence across all herds ranged from 0.0% to 6.2% (median: 0.0%). If UCD was present in a herd, the median within-herd prevalence ranged from 0.9% to 6.2% (median: 3.2%).

Risk Factors for Teat Warts

Herd-Level Risk Factors. At the herd level, conventional milking ($P = 0.02$) and poor teat end condition ($P < 0.01$) were associated with the presence of teat warts on a farm (Table 2). Herds milking with conventional milking systems had almost 5 times the odds of having teat warts in a herd compared with AMS herds. In herds with more than 10% of the cows with a teat end condition score greater than or equal to 3, the odds for teat warts were higher than in herds where no cow had a score greater than or equal to 3.

Cow-Level Risk Factors. A total of 2,359 cows were in 73 teat-wart-positive herds. Because of missing data, only 2,260 cows were included in the final cow-related risk factors model.

At the cow level, univariable mixed-effect regression analysis was first performed for 8 variables (Table 3). Of these, 5 variables (breed, milk yield, cow SCC, teat end condition score, and udder hygiene score) had a $P < 0.25$ and were initially included in the model. After backward selection, only the variable cow SCC ultimately remained in the model ($P < 0.01$, Table 4). Cows with lower SCC also had lower odds of having teat warts than those with SCC > 100,000 cells/mL.

When considering all cows—that is, also those from farms without teat warts—the variables breed and cow SCC remained in the final cow-related risk factors model (results not shown). Brown Swiss cows [odds ratio (**OR**): 2.04, 95% CI: 1.21 to 3.42, $P < 0.01$] and cows of other breeds had increased odds for teat warts compared with Simmental cows (OR: 2.00, 95% CI: 1.16 to 3.49, $P = 0.01$) and cows with low SCC (<100,000 cells/mL) had lower odds compared with cows with SCC > 100,000 (OR: 0.69, 95% CI: 0.53 to 0.89, $P < 0.01$).

Risk Factors for UE

Herd-Level Risk Factors. At the herd level, only one variable was associated with the presence of UE in herds. Herds with a good depth (>5 cm) of bedding

Table 3. Summary of variables screened for association with udder disease (warts, udder edema, udder thigh dermatitis, udder cleft dermatitis) at cow level

Udder disease	Screened variables
Teat warts	Breed,[1] lactation number,[1] DIM,[1,2,3] cow-individual milk yield,[1,3] SCC,[1,3] teat end condition score,[4] UHS,[5,6] LHS[5,7]
Udder edema	Breed, lactation number, DIM, cow-individual milk yield, milk protein concentration[1,3] (%), milk urea concentration[1,3] (mg/dL)
Udder thigh dermatitis	Breed, lactation number, DIM, cow-individual milk yield, UHS, LHS
Udder cleft dermatitis	Breed, lactation number, DIM, cow-individual milk yield, cow SCC, UHS, LHS, hock lesion score[4]

[1]These data are taken from the latest DHIA test day and represent only 1 mo.
[2]Dichotomized as ≤60 DIM and >60 DIM.
[3]Classification based on percentiles.
[4]Score 1 to 3.
[5]Score 1 to 4, dichotomized into clean (scores 1 and 2) and dirty (scores 3 and 4).
[6]UHS = udder hygiene score.
[7]LHS = leg hygiene score.

material for dry cows had reduced odds (OR: 0.22) of UE compared with those with poor bedding depth (Table 2).

Cow-Level Risk Factors. A total of 1,365 cows that came from 40 herds where UE was present were included in the cow-level risk factors analysis.

Six predictor variables were tested in a univariable mixed-effect logistic regression analysis at cow level (Table 4). Four of these variables (lactation number, DIM, cow milk yield, and milk protein concentration) had $P < 0.25$ and were included in a multivariable logistic regression model for cow-level risk factors. The variable DIM remained in the model (Table 4). Cows at the beginning of lactation (d 0 to 60) had 4.9 the odds to experience UE compared with cows in later lactation.

Risk Factors for UTD

Herd-Level Risk Factors. No herd-level risk factor could be identified.

Cow-Level Risk Factors. The 10 UTD-positive herds had a total of 514 cows (512 could be included in the analysis). Six variables were tested for associations with UTD at cow level in univariable mixed-effect regression analysis (Table 4). Of these variables, only one had a $P < 0.25$ (lactation, $P < 0.05$) and therefore remained in the final model. First-lactation animals had almost 4 times the odds to get UTD compared with older cows (Table 4).

Risk Factors for UCD

Herd-Level Risk Factors. Two variables were associated with the occurrence of UCD at herd level (Table 2). Herds with freestalls had 12.6 times the odds of having UCD cases compared with tiestall herds. The odds of having UCD in the herd were increased if dry cows were housed on comfort rubber mats compared with plain rubber mats and no rubber mats for dry cows at all.

Cow-Level Risk Factors. Of all cows, 692 were from 15 UCD-positive herds. Eight variables were evaluated as possible risk factors for UCD in univariable mixed-effect regression analysis (Table 4). Three variables (breed, udder hygiene score, and leg hygiene score) had $P < 0.25$. After backward selection, none remained in the final model.

DISCUSSION

The study was conducted in Bavaria, where almost one-third (28.2%) of German dairy cows are located (Statistisches Bundesamt, 2020). The herd structure of Bavaria varies from that of other German federal states in many ways. For example, the Bavarian-wide herd milk yield was more than 900 kg below the nationwide average at the time of study (LKV Bayern, 2018). This is probably related to the fact that the main breed used was Simmental, a dual-purpose breed. In contrast to other regions of Germany, in Bavaria the Holstein, a pure dairy breed, is the predominant breed (PraeRi, 2020). Herd size is also more than 40% smaller than the national average (LKV Bayern, 2018), and tiestalls are more common compared with other German regions. Therefore, these findings should not be generalized for all of Germany and only represent larger Bavarian dairy herds, as we excluded very small farms with fewer than 10 cows (17% of all farms).

This the first study to investigate the prevalence of teat warts, UE, UTD, and UCD and their risk factors in Germany. Because of the cross-sectional nature of

Table 4. Results of multivariable mixed logistic regression analyses at cow level for cow-related risk factors associated with the udder diseases teat warts (n = 2,260), udder edema (n = 1,365), and udder thigh dermatitis (n = 512), including only cows from herds with the disease present (herd included as random effect)

Disease	Variable	n[1]	Coefficient	SE	OR[2]	95% CI	*P*-value
Teat warts							
	Intercept		−1.76	0.16			<0.01
	Cow SCC (cells/mL)						
	≤100,000	1,340	−0.36	0.13	0.70	0.54–0.90	<0.01
	>100,000	920	Referent				
Edema							
	Intercept		−3.7	0.21			<0.01
	DIM						
	0–60	244	1.59	0.29	4.90	2.79–8.66	<0.01
	>60	1,121	Referent				
Udder thigh dermatitis							
	Intercept		−4.49	0.50			<0.01
	Lactation						
	1	153	1.28	0.65	3.62	1.00–13.07	<0.05
	≥2	359	Referent				

[1]Number of cows per category.
[2]OR = odds ratio.

the study, short-lived diseases or those associated with a particular stage of lactation (e.g., UE) will likely be underrepresented. However, the aim of the study was to provide a first estimate of the apparent prevalence of these diseases in Bavaria, which we achieved.

Prevalence and Risk Factors for Teat Warts

Teat warts were the most commonly found disease in this study. As teat warts can persist for several months (Ohnstad et al., 2007), the likelihood of detecting them in a cross-sectional study is higher than that of detecting shorter-lasting lesions such as UE.

Prevalence data for teat warts are sparse. A retrospective survey study in Egypt found an incidence of 1.65% for teat warts (Nouh et al., 2014), and an older study in Bangladesh found a prevalence of 15.9% (Nooruddin et al., 1997). Neijenhuis et al. (2004) found a prevalence of 21.5% before and 13.4% after changing from conventional to AMS milking in Dutch herds.

The latter findings are consistent with ours, where the odds for teat warts were 5 times higher in herds with conventional milking systems compared with AMS. As the implementation of liner disinfection between cows is more common in AMS, one might assume that the viral transmission via the teat cup liner might be lower. However, this assumption could not be supported with observations in this study, as we were unable to identify associations between milking hygiene parameters (e.g., use of liner disinfection, postdip usage, wearing of single-use gloves during milking, and establishment of milking order) and the prevalence of teat warts.

Herds with poor teat end condition (hyperkeratosis) and cows with higher SCC, an indicator of subclinical mastitis (Dohoo and Meek, 1982), had increased odds for having cows with teat warts in this study. There are several possible explanations for these observations. One is that teat warts could negatively affect the milking process based on poor teat-liner contact and increase the risk of hyperkeratosis and subclinical mastitis due to liner slips (Baxter et al., 1992). Another is that cows with hyperkeratosis and high SCC were more likely to experience a damaged (mucosal) skin barrier or impaired immune system and therefore had an increased susceptibility to papillomaviruses. Although the temporal aspect of this association cannot be answered with a cross-sectional study, skin damage might be a risk factor for teat warts.

Prevalence and Risk Factors for UE

In our study, 1.1% of cows had UE. As per study design, we evaluated cows of all stages of lactation at one point in time, whereas other studies have focused on the high-risk periparturient time period to investigate UE. This explains the much higher UE prevalence reported in studies from the United States (97%; Dentine and McDaniel, 1983) or Canada (70%; Morrison et al., 2018). In our study, cows in early lactation (0 to 60 d) had the highest odds of showing UE, which supports early lactation as a risk factor for UE once more.

At the herd level, a good depth of bedding material for dry cows (means > 5 cm) was associated with UE. Several studies have found that cows prefer soft, compressible lying surfaces (Natzke et al., 1982; Herlin, 1997; Tucker et al., 2003). Whether lying behavior influences lymphatic flow or the development of UE cannot be answered with our data.

Prevalence and Risk Factors for UTD

For UTD, we found the prevalence to be at the low level of 0.2%. Again, because of the study design our reported prevalence was lower than that of other studies. Another German study reported that 1% of heifers and 0.06% of multiparous cows had UTD over a period of 3 years (Sigmund et al., 1980). A French study also had much higher annual UTD incidences of 23% for primiparous and 1.2% for multiparous cows (Roy et al., 2012). Consistent with our results, Roy et al. (2012) also showed that primiparous cows are more frequently affected by UTD compared with multiparous cows. Udder thigh dermatitis usually occurs as a result of friction when the udder is edematous on the inner thigh, and UE also occurs mainly in heifers (Roy et al., 2012; Morrison et al., 2018).

Nevertheless, none of the total 15 UTD-positive cows also had UE in this study. Because UTD usually does not appear until the edema has decreased, UE might have already subsided in the cows affected with UTD in this study. However, it should be emphasized that because of the cross-sectional study design and low number of positive cases (especially for UCD and UTD), the results should be interpreted with caution.

Prevalence and Risk Factors for UCD

The prevalence of UCD, 0.3% of all cows, was clearly below the prevalence in the Netherlands, 5.2% (Olde Riekerink et al., 2014). In Sweden, the prevalence among cows was even higher at 18% (Persson Waller et al., 2014) and 28% (Ekman et al., 2018). Although Persson Waller et al. (2014) found breed to be a risk factor for UCD, breed was not associated with UCD in our study. In the Swedish studies, the Swedish Holstein and Swedish Red breeds were predominantly sampled, whereas Simmental was mostly sampled in our study. This could contribute to this discrepancy.

At the herd level, the use of comfort (padded) rubber mats during the dry period was identified as a risk factor for UCD during lactation. Compared with farms that had plain rubber mats or completely different bedding for dry cows, the odds for UCD were increased. These findings are consistent with the study of Ekman et al. (2018), who found comfort rubber mattresses as a risk factor for UCD compared with normal rubber mats.

Ekman et al. (2018) speculated that comfort rubber mattresses are more likely to cause heat and moisture buildup, and that the mats' deformability decreases over time. Subsequently, feces and urine may accumulate and cause skin barrier damage when the cow is lying down.

Methodological Considerations

The farms were contacted from telephone lists, and participation was voluntary. The question arises as to why a farm agrees to participate or not. In this study, 54% of farms declined. One could speculate that smaller farms are more likely to know what can be found in their herds and therefore do not want to participate.

The prevalence of the diseases was unknown beforehand, and these data were collected as part of a different study. Therefore, no formal sample size calculation was conducted for each disease, but the sample size was deemed meaningful even for these rare diseases. However, despite a low effective sample size for some diseases (including missing DHIA data for some herds), we were able to identify some risk factors.

Furthermore, 20 trained technicians visited the herds and collected the data. Though they are regularly trained on disease identification and sample collection, no interobserver variability was assessed, and misclassification or detection bias will not be completely avoidable. However, the technicians were in teams of 2 on each farm to look for diseases, which should improve the interobserver agreement as unclear cases would have been examined by 2 people who were specifically looking for these lesions.

Disease definitions may vary from those used by other studies. This may result in different detection rates. The diseases are sometimes only vaguely defined in other studies (e.g., Roy et al., 2012; Morrison et al., 2018). For example, Morrison et al. (2018) had an adspectoral scoring system for UE but no more precise definition based on palpation. Both overestimation and underestimation may occur. For example, UE may not be diagnosed visually alone, and palpation to check the consistency of the udder tissue is an important part of the diagnosis. Some teat warts could be mistaken for udder pox, but this could have only been confirmed with absolute accuracy by laboratory analysis.

Seasonal influences were not considered in our study. The majority of farms (n = 117) were visited between March and May, including 73 farms in April alone, and none in midsummer (July or August). For this reason, risk factors influenced by season (e.g., grazing) were ignored.

CONCLUSIONS

This is the first study in southern Germany about the prevalence and risk factors of various udder diseases other than mastitis—namely, teat warts, UE, UCD, and UTD. The diseases were rarely detected in the study cows. The most common disease was teat warts (in 4.0% of all cows). The risk factors identified for teat warts were conventional milking on the herd level and cow SCC on the cow level. Poor depth of bedding material was associated with UE on the herd level and early DIM on the cow level. Freestall housing and the use of comfort rubber mats in the dry period were identified as risk factors for UCD on the herd level. Cows in first lactation had increased odds for UTD compared with cows in later lactations. Further studies that examine each disease in depth should identify additional risk factors.

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2. Publication II

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Mastitis pathogens in Bavaria, Southern Germany: apparent prevalence and herd-level risk factors

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Mastitis pathogens in Bavaria, Southern Germany: apparent prevalence and herd-level risk factors

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Abstract

The aim of this cross-sectional study was to determine the prevalence of mastitis pathogens in Bavaria and to identify management practices as possible risk factors for the presence and within-herd prevalence of the four most common pathogens.

For this purpose, aseptic quarter milk samples of 6,188 dairy cows on 152 Bavarian dairy farms were collected and a California mastitis test was performed. Udder and leg hygiene as well as teat end condition were scored at cow-level. Teat end hygiene after udder preparation was evaluated for about ten cows per herd. Herd information and information on management practices were obtained using a standardized checklist. Microbiological analyses were carried out according to the guidelines of the German Veterinary Medical Society at the laboratory of the Bavarian Animal Health Service e.V. To determine herd-level risk factors, Fisher's exact test for categorical, and Student's t-test or Mann-Whitney-U test for continuous data were performed. In addition, multivariate logistic regression was performed to detect risk factors for the presence of pathogens in the herd and a multifactorial Poisson regression analysis was done to assess for the association of risk factors with within-herd prevalence.

The most frequently detected pathogens at quarter-level were CNS (4.4%), *Staphylococcus aureus* (2.9%), *Streptococcus dysgalactiae* (0.9%), and *Streptococcus uberis* (0.9%). Each of these four pathogens was detected in more than half of the herds (90%, 70%, 61%, and 54%, respectively). Freestall housing and larger herds were associated with the detection of CNS and *Streptococcus uberis*. The usage of post-milking teat disinfection was associated with a lower within-herd prevalence of *Staphylococcus aureus*. The use of internal teat sealants and blanket dry cow therapy reduced the odds for detection of *Streptococcus dysgalactiae* at the herd-level. However, the latter practices were implemented by only a minority of herds. The study shows for the first time the apparent prevalence of mastitis pathogens in Bavaria using a sample that is not derived from submissions to a diagnostic laboratory. CNS were found to be the most frequently isolated pathogens, further studies on the etiology and reduction of these pathogens should be considered.

Key words: *mastitis pathogens, prevalence, cross-sectional study, management practices*

Introduction

Mastitis is considered the most important disease in the dairy sector in many aspects. Besides the potential risk for food safety [1, 2], mastitis has major economic consequences (including treatment, downtime, and penalty costs) for dairy farms as well as a considerable impact on animal welfare [3, 4]. Therefore, it is necessary to monitor the prevalence of mastitis pathogens, to observe trends and to decide the appropriate control measures. Since the late 1960s, the 5-point plan for the control of mastitis has been implemented. The aim of this plan was to reduce mastitis, mainly by controlling contagious pathogens through consistent implementation of management practices, such as milking machine maintenance, teat dipping, treatment of clinical mastitis cases, antibiotic dry cow therapy, and culling of chronically diseased animals [5]. As a result, the prevalence spectrum of mastitis pathogens has changed from primarily cow- to primarily environment-associated pathogens [6]. The proportion of environmental pathogens (such as *Escherichia* **(E.)** *coli*, *Streptococcus* **(Str.)** *uberis*) has increased with decreasing prevalence of contagious pathogens (such as *Staphylococcus* **(S.)** *aureus*, *Str. agalactiae*). Nevertheless, a recent worldwide meta-analysis found that the most common pathogens detected in milk include *S. aureus*, coagulase-negative staphylococci **(CNS)**, *E. coli*, *Str. agalactiae*, and *Str. uberis* [7].

Although reports of the Bavarian Animal Health Service on the prevalence of mastitis pathogens are available, these reports are based on submissions to a diagnostic laboratory and are therefore biased [8, 9]. They are most likely an overrepresentation of quarter milk samples of herd screenings of farms with particularly high bulk tank somatic cell or bacterial counts and/or quarter milk samples of individual cows with mastitis. An unbiased prevalence estimate was needed. Also, the studies mentioned above did not look for potential risk factors. Although there are already many studies investigating associations of specific management practices or cow factors with the occurrence of mastitis and mastitis pathogens, it is important to identify region-specific associations for particular regions, such as Bavaria with its relatively small Simmental herds. This can contribute to a targeted risk assessment and risk prevention.

Therefore, the aims of the study were to determine the prevalence of mastitis pathogens in Bavaria, Southern Germany, and to identify management practices as potential risk factors for the presence and

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within-herd prevalence of the four most common pathogens.

Materials and Methods

This cross-sectional study was conducted between October 2017 and June 2018. The basis for the recruitment of the herds was a list of all Bavarian dairy farms (n=28,884). Assuming that herds with less than 200 kg milk production per day had less than ten dairy cows, these herds were excluded (n=4,873). The remaining farms (n=24,011) were then divided into four groups based on the quartiles of daily shipped milk (in kg; group 1: 200-378; group 2: 379-619, group 3: 620-1079, group 4: 1080-40704). Per group, 200 herds were randomly selected. With the aim to recruit 40 herds per group, these lists were distributed to the ten branches of the Bavarian Animal Health Service with potential herds for their respective region. The total number of 160 herds was set due to budgetary limitations but was deemed to provide sufficient information. The technicians of the Bavarian Animal Health Service contacted the farms by phone along this provided list. Each farm was visited once by trained technicians. In total, a maximum of 100 cows were examined per farm; for larger farms (>100 cows), 100 cows were randomly selected beforehand based on cow lists provided by farmers. At milking, each cow was evaluated for udder hygiene (score 1-4 [10]), leg hygiene (score 1-4 [11]), and teat end condition (score 1-4 [12]). For the teat end condition score, all four teats were assessed, but only the highest score per cow was recorded. To assess the cleanliness of the teat ends after the precleaning by the milker, the Bavarian Animal Health Services technicians swiped the teat ends from about ten cows with an alcohol wipe before cluster attachment. The wipes were scored based on the scoring system by Cook and Reinemann [13]. Then a California Mastitis Test (**CMT**, DeLaval Holding AB, Tumba, Sweden; 0, 1, 2, 3, corresponding to -, +, ++, +++ after Barnum and Newbould, 1961 [14]) was performed for each cow and quarter. Aseptic quarter milk samples were collected according to the German Veterinary Association standards [15] in sterile sample tubes with boric acid as conserving agent and cooled immediately. On farms with post-milking teat disinfection **(PMTD)**, the average teat coverage with the teat-dip was assessed after milking (<20%, 20-50%, >50% covered). The aseptic quarter milk samples were transported to the laboratory of the Bavarian Animal Health Service central in Grub, where either on the same day or the next morning, they were analyzed for mastitis pathogens according to the German Veterinary Association guidelines [15].

One plate per cow was prepared with inoculation loops on a non-selective nutrient medium (aesculin blood agar with sheep blood added; Oxoid Deutschland GmbH, 46483 Wesel) with 0.01 mL milk per quarter. The plates were then incubated at 36 ±1 °C and first assessed after 18 to 24 hours. First, they were examined and differentiated by their colony morphology, gram stain as well as the formation of hemolysis zones in streptococci and hemotoxin zones in staphylococci. *S. aureus* was assumed to be isolated with positive coagulase and clumping factor as a means of differentiation from CNS. Using MALDI-TOF MS (microflex MALDI Biotyper, reference database V.3.3.1.0., Bruker Daltonik GmbH), a subset of CNS per herd (mostly from clinical quarters) were further differentiated into the individual species (e.g., *S. haemolyticus*, *S. chromogenes*, *S. epidermidis*). In case of non-assignment to the pre-set CNS species or no further differentiation, the reports were indicated with "Staphylococcus (CNS)".

For further differentiation of streptococci, haemolysis patterns and aesculin hydrolysis ability were tested. In addition, the CAMP test was performed to differentiate between the aesculin-negative streptococci *Str. agalactiae* (CAMP-test positive) and *Str. dysgalactiae* (CAMP-test negative). To differentiate *Str. dysgalactiae* from *Str. canis*, Lancefield groups were determined (*Str. dysgalactiae* group C, *Str. canis* group G). For more precise differentiation of Enterobacteriaceae, they were grown on Gassner agar (Merck KGaA, 64293 Darmstadt) to test for lactose conversion.

In addition to CNS, more accurate differentiation was also performed for gram-negative rod bacteria and aesculin-positive streptococci using MALDI TOF MS. Aesculin-positive streptococci were classified as *Str. uberis*, enterococci (*E. faecalis*, *E. faecium*), lactococci (*L. lactis*, *L. garviae*), and if not classified into either of these, they were reported as aesculin-positive streptococci. Contaminated samples (≥3 bacterial species) were aggregated with the "no growth" samples during analysis, when the percentage of samples with pathogens were calculated.

Visual secretory changes like flakes, clots or the occurrence of pus or blood were defined as clinical mastitis **(CM)**. Subclinical mastitis **(SUBM)** was defined as positive CMT result (i.e., ≠ 0, but visually normal milk).

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NY, USA). For descriptive analyses of quarter-level, herd-level, and within-herd pathogen prevalence as well as herd data and management practices, PROC FREQ was used for a description of categorical and PROC MEANS for continuous data, respectively. The herds were divided into four groups by increasing cow number using the call lists: group 1 (n=38; 12-26 cows), group 2 (n=37; 27-40), group 3 (n=39, 41-61), and group 4 (n=38; 62-327).

The association of herd factors and management practices with

Table 1: Farm-level variables investigated for association with the presence of Coagulase-negative Staphylococci (CNS), *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*, respectively.

Group of variables	Variables
General herd factors	Herd size, group[1], predominant breed (Brown Swiss/ Simmental/ other), operating structure (conventional/organic), rolling herd average milk production[2], bulk tank somatic cell count[3] (BTSCC), bulk tank bacterial count[3] (BTBC), teat end condition score[4], leg hygiene score[4] (LHS), udder hygiene score[4] (UHS), teat end hygiene[4], open herd[5], farming areas (dairy and crop production/ dairy, crop and beef production/dairy production and youngstock/ dairy and beef production and youngstock/ dairy production only)
Dry cow management	Abrupt cessation[5], intermittent cessation[5], blanket dry cow therapy[5], use of external teat sealants[5], use of internal teat sealants[5], group formation for drying-off[5]
Milking routine	Milking (conventional/milking robot), precleaning method (one paper per cow/ one cloth for several cows/ other), post-milking teat disinfection (PMTD, all animals/ none), single use gloves[5], fixed milking order of cows[5], dip coverage (<20%/ 20-50%/ >50%)
Housing	Stall type (tiestall, freestall), bedding material (lime-straw/ straw-hay/ sawdust/ none/ other)

[1] Herds divided into four groups based on call list: group 1 (12-26 cows), group 2 (27-40), group 3 (41-61), group 4 (62-327)
[2] Calculated by dividing the herd milk production over one year by the average number of animals tested per day
[3] Geometric mean of the preceding three months at the time of the visit
[4] Scoring from 1-4, proportion of cows with score 3+4 per herd
[5] (Yes/No)

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the herd-level presence of a pathogen as well as within-herd prevalence were assessed. Therefore, the four pathogens CNS, *S. aureus*, *Str. dysgalactiae* and *Str. uberis* were all four tested for the same set of variables. The variables investigated for association are listed in Table 1. Exact Fisher test was used for categorical and Student's t-test or Mann-Whitney-U test (PROC NPAR1WAY) for continuous data. Significance level was set at α = 0.05. Variables that were associated with the herd-level prevalence of a mastitis pathogen were tested in a multivariate logistic model (PROC LOGISTIC) and were eliminated through manual backward selection. Potential factors associated with the within-herd-prevalence of each mastitis pathogen were tested in multifactorial Poisson regressions using PROC GENMOD. Through backward selection (P>0.05) the most parsimonious model was identified. Mean and variance of each pathogen's prevalence were compared and if the variance of the pathogen distribution was greater than the mean, to evaluate if a Poisson analysis was preferred. Additionally, the overall fit of each model was assessed by evaluating residual plots and whether overdispersion was present or not to decide whether a Poisson or a negative binomial distribution was suited best for the data.

Results and Discussion

Study population: The response rate for the study was 46%. Four farms rescinded their participation shortly before the herd visit and in the end 152 farms were visited. The total herd size ranged from 12 to 326 cows, with a mean of 48 (SD: ± 33). Only three farms had more than 100 cows (111, 121 and 327 cows, respectively). The majority of farms were member of the regional Dairy Herd Improvement Association (86%), produced conventionally (88%), and housed their cows in freestall barns (59%). Conventional milking systems (91%) were more prevalent than automatic milking systems (9%).

Most farms had Simmental (76%) or Brown Swiss cows (13%) as predominant breed. The median rolling herd average milk production was 7,906 kg/year (interquartile range, **IQR**: 6,884-8,626). The bulk tank somatic cell **(BTSCC)** and bacterial counts averaged (median) 147,100 cells/mL (IQR: 107,000-190,000 cells/mL) and 14,000 cfu/mL (IQR: 8,000-23,000 cfu/mL), respectively.

In the assessment of hygiene at herd-level, poor hygiene scores (scores 3-4) were found for 14% (median; IQR: 4-32%) of the cows for udder hygiene and even for 42% (median; IQR: 15-66%) of the cows for leg hygiene.

When evaluating teat hygiene after pre-cleaning by the milker, a median of 36% (IQR: 13-58%) of the assessed cows were still found to have inadequate hygiene, i.e., score 3 or 4. On the contrary, poor teat end condition (score 3 or 4) was found in only a median of 1% (IQR: 0-9%) of the cows in the herds.

The average herd size of this study was slightly higher than the actual average (42 cows/herd in Bavaria according to the German Federal Statistical Office in 2020 [16]), because of the exclusion of small herds with less than 10 cows. Nevertheless, Bavaria's herd structure differs to herd structure in other German federal states. The national average herd size of German dairy herds is larger (with an average of 196 cows per herd in the eastern German states and 60 cows per herd in the western German states [16]), herds are more likely to use freestall housing and are more likely to have Holstein Friesian cows instead of Simmental than the herds in Bavaria [16].

Mastitis prevalence: For simplicity, apparent prevalence will be called prevalence in the following text. In total, 24,360 quarter milk samples of 6,188 cows were collected. Six percent of cows (n=378) had non-milking ("dry") quarters (n=392). A third of cows (32%) had at least one quarter affected with either SUBM or CM. SUBM was diagnosed in 3,517 (14%) and CM in 158 (0.6%) quarters. The remaining 20,685 quarters (85%) were considered healthy as they showed neither

Table 2: Prevalence of mastitis pathogens in all aseptic quarter milk samples and prevalence within pathogen-positive samples (n=2,655), samples from healthy[1] quarters (n=20,685), subclinical mastitis (SUBM, n=3,517), and clinical mastitis quarters (CM, n=158).

	Samples			Health Status of Quarter		
	all		pathogen-positive	healthy[1]	SUBM	CM
Pathogen	n	%				
Coagulase-negative staphylococci	1073	4.4	40.4	3.0	12.3	6.3
Staphylococcus aureus	713	2.9	26.9	1.9	8.4	13.9
Streptococcus dysgalactiae	228	0.9	8.6	0.3	4.4	9.5
Streptococcus uberis	220	0.9	8.3	0.2	4.6	13.3
Lactococcus spp.	133	0.6	5.0	0.3	2.3	0
Enterococcus spp.	118	0.5	4.4	0.2	2.1	0.6
Other aesculin-positive streptococci	56	0.2	2.1	0.1	1.0	0.6
Streptococcus canis[2]	46	0.3	1.7	<0.1	0.5	16.5
Streptococcus agalactiae	51	0.2	1.9	0.1	0.7	2.5
Trueperella pyogenes	18	<0.1	0.7	<0.1	0.2	2.5
Other Coliforms[3]	13	<0.1	0.5	0	0.3	0.6
Escherichia coli	13	<0.1	0.5	<0.1	0.2	2.5
Other gram-negative pathogens[4]	11	<0.1	0.4	0	0.2	2.5
Other gram-positive pathogens[5]	8	<0.01	0.3	0	0.1	1.9

[1] Neither positive California mastitis test nor visual milk changes regardless of the microbiological findings
[2] Of these 46 positive samples, 44 were due to an outbreak in one herd
[3] *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Citrobacter*
[4] *Serratia marsescens*, *Mannheimia haemolytica*, *Pseudomonas aeruginosa*
[5] Coryneforms, yeast, other aesculin-negative streptococci, *S. hyicus*

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SUBM, based on CMT, nor signs of CM. Cows with SUBM were present in almost all herds (99%, n=150). The median within-herd prevalence for SUBM was 32% (min-max: 0-79%). In 15% of the herds, more than half of their cows had SUBM. In contrast, CM was present in 43.4% (n=66) of herds. The median within-herd prevalence was 0% (min-max: 0-29%) and less than 3% of CM in 75% of the herds.

Quarter level pathogen prevalence: A total of 210 samples (0.8%) were contaminated and were counted with pathogen-negative samples. Microbiological analyses showed that 10.9% (n=2,655) of all collected quarter milk samples were positive for at least one pathogen and 89.1% (n=21,705) had no-growth. Table 2 presents all bacteriological results of the quarter milk sample analyses. Among the positive samples, the most isolated pathogens were CNS (40.4%), *S. aureus* (27%), *Str. dysgalactiae* (8.6%), and *Str. uberis* (8.3%). In a previous report of the Bavarian Animal Health Services, quarter milk samples of herd screenings were evaluated [9]. Comparing the results of the report with the results of the present study, one can observe that all mastitis pathogens (except CNS) were isolated more frequently. *S. aureus* was isolated in 29%, *Str. dysgalactiae* in 10%, *Str. uberis* in 17%, and *Str. agalactiae* in 5% (here: in 2%) of the pathogen-positive samples, respectively. Usually, udder health technicians of the Bavarian Animal Health Services are called to farms that requested quarter milk samples of the herd due to high bulk tank cell counts, bacterial counts, or similar. This likely explains the discrepancy in the results between this study and the report of the Bavarian Animal Health Services in 2017 [9].

Also, among all samples, CNS (4%) were the most commonly isolated pathogens, followed by *S. aureus* (3%), *Str. dysgalactiae* (0.9%), *Str. uberis* (0.9%), and *Lactococcus* spp. (0.6%). Compared to other German studies, we have found partly lower prevalences for CNS and *S. aureus*. The prevalences for CNS and *S. aureus* were slightly higher in a study in Brandenburg [17], at 9% and 6 % of all samples, respectively, or in a study in Hesse [18] at 17% and 5% of all samples, respectively. While the prevalence of *Str. uberis* was similar to that previously found in Brandenburg (1.0%) [17], it was much more common in quarter milk samples from Hesse (9%) [18]. For *Str. dysgalactiae*, the prevalence was at a very similarly low level in both studies mentioned (1.0 and 0.8%, respectively). However, the Hessian study was based on results from a diagnostic laboratory. Similar to the Bavarian Animal Health Services [9], this laboratory was more likely to process samples from farms with milk quality problems. Thus, in the Hessian study [18], 17% of the quarter milk samples came from farms with severe udder health problems. In contrast, a study from Brandenburg [17] sampled only clinically healthy cows from 80 herds. The difference in sample selection prohibits an exact comparison of the studies. In addition, those studies were conducted several years prior to this study and in different regions of Germany with different management practices and breeds. All these aspects will likely explain the slightly different results.

As expected, the likelihood of pathogen isolation (Figure 1) as well as the distribution of pathogens differed by clinical status of the quarter. In healthy quarters or SUBM-samples the most common pathogens were CNS (3.0 % and 12.3%, resp.) and *S. aureus* (1.9% and 8.4%, resp.). In samples with CM, *Str. canis* was the most isolated pathogen (16.5%), followed by *S. aureus* (13.9%), *Str. uberis* (13.3%) and *Str. dysgalactiae* (9.5%).

The Bavarian Animal Health Services reported in 2021 [8] that 44% of samples with SUBM were pathogen-positive, compared to only 36% in the present study. For samples with CM the difference was less pronounced (76% samples pathogen-positive in the report versus 72% samples pathogen-positive in the present study). Their samples [8] were a mixture of individual submissions (usually diseased cows) and samples from herd investigations (often herds requesting an examination due to high cell counts, bacterial counts, or similar). In contrast, this study sampled all cows (few exceptions) from randomly selected herds. Sampling such farms as well as submission behaviour might influence the pathogen detection.

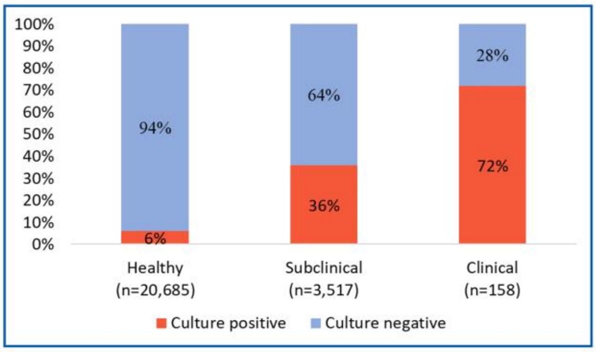

Figure 1. Proportion of aseptic quarter milk samples of 6,188 Bavarian study cows with pathogen detection by clinical status of the quarter. Healthy was defined here as samples from quarters with a negative California mastitis test (CMT) and without visual milk changes.

S. aureus and *Str. uberis* were isolated as the most common CM pathogens in our study, consistent with the Bavarian report [8]. *Str. uberis* turned out there to be the most important pathogen of CM over the years. Similar to our study, in CM cases, *S. aureus*, *Str. uberis*, and *Str. dysgalactiae* were identified among the most commonly isolated pathogens (21.3%, 11.1%, and 15.6%, respectively) by a Swedish study [19]. In this Swedish study, field veterinarians collected samples from cows with acute clinical mastitis. The second most common pathogen of CM there was *E. coli* (15.9%), unlike in our study, where *E. coli* was isolated in only 3% of CM samples (n=4). Since *E. coli* mastitis often has a short, acute to peracute course, the elimination of the pathogen from the udder occurs rapidly [20]. Therefore, the likelihood of detecting pathogens of short-term infections in a cross-sectional study is lower than detecting pathogens of infections that persist over a longer period. Furthermore, *E. coli* can cause very severe mastitis with recumbency [21] and recumbent cows would unlikely be milked with the rest of the herd, when our samples were taken.

The high isolation risk of *Str. canis* in the present study was attributable to an *Str. canis* outbreak in a single herd and should therefore not be overinterpreted. Of 78% (n=14) cows with CM in that herd, 14 CM quarters were infected with *Str. canis*. Usually, intramammary infections with *Str. canis* results in a considerable increase in somatic cells [22], albeit the mastitis tends to remain subclinical. This study showed for the first time that *Str. canis* can also lead to an actual CM outbreak. However, the high proportion of *Str. canis* isolates due to one herd outbreak also highlighted that the sample size of this scoping study was limited, and the precision of the estimates has to be considered to be fairly low.

Herd level prevalence and risk factors: Although only up to 100 cows were sampled per herd, we will refer to the whole herd below, because only three of the 152 farms had more than 100 cows. In the following, only risk factors with significant associations will be further addressed. A herd was considered positive for a pathogen if this pathogen was isolated in at least one quarter. An overview of herd prevalence and

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Table 3: Herd-level and within-herd prevalence of mastitis pathogens in 152 study herds in Bavaria, Southern Germany[1].

	Herds positive		Within-herd prevalence (%)		
Pathogen	(n)	(%)	Median	Min	Max
Coagulase-negative staphylococci	136	89.5	11.8	0	40.9
Staphylococcus aureus	107	70.4	4.2	0	92.6
Streptococcus dysgalactiae	92	60.5	2.7	0	24.0
Streptococcus uberis	82	54.0	1.5	0	21.1
Lactococcus spp.	23	15.1	0	0	40.7
Enterococcus spp.	45	29.6	0	0	21.2
Other aesculin-positive streptococci	14	9.2	0	0	35.4
Streptococcus canis	2	1.3	0	0	36.5
Streptococcus agalactiae	5	3.3	0	0	26.5
Trueperella pyogenes	15	9.9	0	0	4.4
Other coliforms[2]	8	5.3	0	0	4.7
Escherichia coli	12	7.9	0	0	6.9
Other gram-negative pathogens[3]	10	6.6	0	0	4.6
Other gram-positive pathogens[4]	7	4.6	0	0	5.3

[1] In herds with >100 cows (n=3; 111, 121 and 327 cows, resp.) only 100 cows were sampled
[2] *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Citrobacter*
[3] *Serratia marsescens*, *Mannheimia haemolytica*, *Pseudomonas aeruginosa*
[4] Coryneforms, yeast, other aesculin-negative streptococci, *S. hyicus*

within-herd prevalence for all mastitis pathogens can be found in Table 3.

CNS were found in 90% of herds (n=136) and also the median within-herd prevalence was highest for CNS with 11.8% (min-max (%): 0-40.9). In 10 herds, more than 30% of the cows were found positive for CNS. This is similar to other studies, where CNS were among the most commonly detected pathogens in milk samples [17, 18, 23]. In the study from Brandenburg [17], CNS were detected evenly in all 80 herds.

Results of univariable analysis showed that herds with Simmental or with Brown Swiss as the predominant breed had a lower within-herd prevalence of CNS, 12% and 14%, respectively, compared with herds with other breeds (19%, P=0.02). This is in agreement with the finding of associations between breed and mastitis incidence in several studies [24–26] . However, these studies also investigated other breeds than in our study (for example, Swedish Holstein and Swedish Red). Possible explanations for breed-related differences include differences in innate mastitis resistance, varying efficiency of immune defences, and also factors such as higher milk production of certain breeds, such as Holstein Friesian, which may be associated with an increased susceptibility to diseases such as mastitis [25]. Interestingly, of the open herds, i.e., herds with external purchases, only 76% had CNS and were therefore less frequently affected compared with herds that did not make external purchases (92%, P = 0.05). Herd size and housing type were associated with the presence of CNS on farms. Herds with freestall housing had 3.5 times higher odds to have CNS than herds with tiestalls (P=0.03, 95% CI:1.17-10.81). Also, CNS were more frequently detected in large herds (P<0.01): the median number of cows in herds, where CNS were detected, was 50 (IQR: 28-62) versus 29 (IQR: 7-33), where CNS were not detected (P<0.05). CNS are part of the commensal microbial flora of the teat skin [27]. Milk leakage can lead to contamination of the housing [28]. Therefore, one can speculate that in freestalls the possibility of transmission between cows is higher than in tiestalls. Since freestall housing was predominantly found in larger herds, herd size might be the surrogate factor. When performing the logistic regression analysis, only the variable housing type ultimately remained in the model (Table 4), confirming the results of the univariable analysis.

In the final Poisson regression model (Table 5), the variable breed remained significant. In herds with predominantly Simmental cows, the number of cows infected with CNS decreased compared to herds with other breeds (P<0.01).

S. aureus was found on 70% of herds (n=107). The within-herd prevalence averaged (median) at 4% (IQR: 0 – 9.0%). In herds where PMTD was practiced, the within-herd prevalence of *S. aureus* was 4%. In contrast herds, that did not use PMTD, had an average within-herd prevalence of 11% (P<0.01). *S. aureus* is a contagious skin pathogen [29] and PMTD is a well-known control/prevention strategy for *S. aureus* infections [30–32]. Nevertheless, more than half of the farms of this study (53%) did not use any PMTD. Poisson regression analysis also showed that PMTD was associated with within-herd prevalence: the number of cows infected with *S. aureus* increased by 1.0 without PMTD (P<0.01, Table 5).

Furthermore, in the multivariate logistic regression model, the variable group formation for drying-off was associated with the presence of *S. aureus* in the herd (Table 4). In herds with such group formation, the odds for *S. aureus* were higher than in herds without separate group formation for drying-off (P=0.02, OR: 4.27, 95% CI:1.22-15.00). Only 18% of the herds in this study reported separating their cows into groups for the dry period. Basically, this approach splits the dry cows into close-up and far-off dry cows. The grouping allows for a targeted adjustment of feeding management to energy requirements [33]. Feeding has an impact on metabolic disorders, such as ketosis and acidosis, which decrease the activity of immune defence cells, which can lead to an increased risk of infectious diseases such as mastitis [34]. In this study, there seems to be no positive relationship with the presence of *S. aureus* in the herd. However, more detailed information on group formation for drying off, such as social grouping, BCS, was not asked.

Str. dysgalactiae was found in 60.5% (n=92) of all herds. The median within-herd prevalence was 2.7% (min-max: 0-24.0%). *Str. dysgalactiae*-negative farms had a median BTSCC of 133 (x1,000 cells/ml; IQR: 97-154), whereas farms with *Str. dysgalactiae*-infected cows had a higher BTSCC with a median of 161 (IQR: 117-203, P<0.01). In CNS-positive herds the median BTSCC was 150 (IQR: 110-195), in *S. aureus*-positive herds and *Str. uberis*-positive herds 151 (IQR: 113-198, and 116-203, respectively). Of the herds without any bedding or with sawdust, 76% and 70% respectively had *Str. dysgalactiae* in the herd and were therefore more often affected (P=0.01) compared to herds with lime-straw bedding (45% positive for *Str. dysgalactiae*), straw-hay bedding (53% positive), and other bedding material (40% positive).

Two risk factors associated with the presence of *Str. dysgalactiae* in a herd were found to be related with drying-off management. Herds that used internal teat sealants at drying off had lower odds of infection compared to herds that did not use them (OR: 0.40, 95% CI: 0.18-0.91, P=0.04). Similarly, the use of blanket dry cow therapy reduced the odds of *Str. dysgalactiae* infection of a herd (0.37; 0.18-0.75, P=0.01).

Table 4: Results of the multivariate logistic regression analysis at herd-level for risk factors associated with the presence of the pathogens Coagulase-negative staphylococci (CNS), *Staphylococcus (S.) aureus*, *Streptococcus (Str.) dysgalactiae*, and *Str. uberis*, respectively.

Pathogen	Parameter		Estimate	SE[1]	OR[2]	95% CI[3]	P-value
CNS		Intercept	2.19	0.28			<0.01
		Housing type					
		Tiestalls	-0.63	0.28	0.28	0.09-0.86	0.03
		Freestalls	Referent				
S. aureus		Intercept	0.67	0.19			<0.01
	Group formation for drying-off						
		Yes	1.45	0.64	4.27	1.22-15.00	0.02
		No	Referent				
Str. dysgalactiae		Intercept	0.06	0.27			0.83
	Usage of internal teat sealants at drying-off						
		No	0.61	0.23	3.38	1.36-8.43	<0.01
		Yes	Referent				
	Blanket dry cow therapy						
		No	0.49	0.20	2.68	1.24-5.77	0.01
		Yes	Referent				
	Bedding material						
		Lime	0.09	0.45	0.25	0.40-1.63	0.84
		Lime-straw	-0.84	0.42	0.10	0.02-0.62	0.05
		Straw-hay	-0.40	0.33	0.16	0.03-0.84	0.22
		Other	-0.93	0.59	0.09	0.01-0.72	0.12
		None	0.62	0.46	0.43	0.07-2.74	0.18
		Sawdust	Referent				
Str. uberis		Intercept	0.03	0.18			0.85
	Housing type						
		Tiestalls	-0.80	0.18	0.20	0.10-0.41	<0.01
		Freestalls	Referent				

[1] Standard Error
[2] Odds ratio
[3] 95% Confidence interval

However, both practices were not widely practiced by the study herds (teat sealant: 20%, and blanket dry cow therapy: 31% of herds). Several studies have attributed beneficial effects to internal teat sealants in reducing intramammary infections, especially in reducing infections with environmental pathogens [35–37]. *Str. dysgalactiae* is classified as both a contagious [38] and an environment-associated pathogen [39]. Thus, internal teat sealants should be recommended for herds with *Str. dysgalactiae*. However, with regard to the critical antibiotic resistance situation, blanket dry cow therapy should not be generally recommended and only be implemented when warranted (e.g., if there is a specific herd problem). Also, in the final logistic regression model the three variables blanket dry cow therapy, internal teat sealants and bedding material remained significant (Table 4).

Similar effects remained in the final Poisson regression model: again, when internal teat sealants were not used and blanket dry cow therapy was not practiced, the number of infected cows increased on farm (P<0.01, Table 5). When considering the types of bedding, it became apparent that compared with sawdust, the number of cows affected with *Str. dysgalactiae* in the herd decreased by at least 0.5 for each type of bedding (Table 5). Sawdust becomes moist quickly, dries poorly – both factors that promote a rapid bacterial growth [40]. In comparison, pure lime bedding and lime-straw performed better as lime increases the pH of the bedding and reduces bacterial growth [41].

Among the esculin-positive streptococci, *Enterococcus* spp. was detected in 29.6% and *Lactococcus* spp. in 15.1% of all herds.

The most important pathogen among esculin-positive streptococci, *Str. uberis*, was found in more than half of all herds (54.0%, n=82) with a median within-herd prevalence of 1.5% (min-max: 0-21.0%). The odds of detecting *Str. uberis* in a herd were 5-fold (95% CI: 2.5-9.9) higher in herds with freestalls compared to herds with tiestall housing. Also, farms in which *Str. uberis* was detected had a median herd size of 52 (IQR: 22-55), whereas herds in which *Str. uberis* was not present had a median herd size of only 30 (33-70; P<0.01). *Str. uberis* can be shed via the intestinal tract and faeces into the dairy environment [42, 43]. A possible explanation could be that cows kept in tiestalls are less able to distribute the contaminated faeces. But also, contagious transmission routes for *Str. uberis* are known [44, 45]. Therefore, as described for CNS, the higher contact between cows in freestalls might also explain the increased risk for *Str. uberis* presence.

Interestingly, Fisher's exact revealed that 63% of herds practicing PMTD had *Str. uberis* in the herd compared to herds without PMTD, where only 45% of herds had *Str. uberis* cases (P<0.01). Post milking teat disinfection is an effective means of reducing contagious and environmental mastitis. The risk of contamination and consequently infection with

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Table 5: Final Poisson regression models for risk factors associated with the within-herd prevalence of the pathogens Coagulase-negative staphylococci (CNS), *Staphylococcus (S.) aureus*, and *Streptococcus (Str.) dysgalactiae*, respectively.

Parameter		Estimate	SE[1]	Wald 95% CI		p-value
CNS	-Scaled deviance: 413.53 on 149 DF[2]-					
Intercept		-1.67	0.08			<0.01
Breed						
	Brown Swiss	-0.15	0.12	-0.39	0.09	0.22
	Simmental	-0.42	0.09	-0.59	-0.25	<0.01
	Other	Referent				
S. aureus	-Scaled deviance: 625.95 on 150 DF-					
Intercept		-3.18	0.08			<0.01
Post-milking teat disinfection						
	No usage	1.00	0.10	0.81	1.20	<0.01
	Usage	Referent				
Str. dysgalactiae	-Scaled deviance: 224.24 on 140 DF-					
Intercept		-3.64	0.31			<0.01
Blanket dry cow therapy						
	No	0.73	0.19	0.37	1.10	<0.01
	Yes	Referent				
Usage of internal teat sealants at drying-off						
	No	0.65	0.21	0.24	1.06	<0.01
	Yes	Referent				
Bedding material						
	Lime	-0.83	0.24	-1.30	-0.35	<0.01
	Lime-straw	-1.18	0.25	-1.68	-0,69	<0.01
	Straw-hay	-1.15	0.23	-1.60	-0.71	<0.01
	Other	-1.45	0.42	-2.27	-0.63	<0.01
	None	-0.65	0.24	-1.13	-0.18	<0.01
	Sawdust	Referent				

[1] Standard Error
[2] Degrees of freedom

environmental pathogens such as *Str. uberis* is higher between milkings due to the widespread distribution in the environment, especially when using only short-lasting dips [46].When conducting logistic regression analysis, except for housing type, no variables remained in the final model (Table 4), poisson regression analysis revealed no significant results.

Lastly, the interpretation of the associations of so-called risk factors with the presence of pathogens in the herd should be done with caution given the study design. The temporal aspect of an association with the implementation of a management practice cannot be answered due to the single point in time for the data collection. For example, a management practice may have been practiced previous to a herd problem, or it may have been newly implemented as a reaction to a herd problem.

Conclusions

In conclusion, the pathogen distribution at quarter- and herd-level in Bavaria differed to some extent from the pathogen distribution in other German federal states and countries. Overall, there is a need for further action to improve mastitis control, as about 32% of the study cows had at least one quarter with CM or SUBM. In SUBM-samples, CNS, *S. aureus*, and *Str. dysgalactiae* were detected most frequently; in CM samples, *S. aureus*, *Str. uberis*, and *Str. dysgalactiae* were commonly isolated. Unexpectedly, *Str. canis* was the most frequently isolated in samples with CM, which was due to one outbreak of *Str. canis* in a single herd. CNS and *Str. uberis* were detected mostly in larger herds and on farms with freestalls. Measures such as post-milking teat disinfection and internal teat sealants reduced the odds for *S. aureus* and *Str. dysgalactiae*, respectively, but were implemented in only a few farms.

Compliance with ethical standards

The authors declare there is no conflict of interest. No ethical approval under the German animal welfare law was required for sample collection.

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IV. DISCUSSION

1. General aspects

Within the framework of this cross-sectional study, an overview of udder health in Bavaria, Southern Germany, could be obtained. The prevalences of teat warts, UE, UTD, UCD, and for mastitis, specifically mastitis pathogens, were determined. In addition, cow factors or management practices, or both, were identified that were associated with the occurrence of these udder diseases.

Udder thigh dermatitis and UCD, in particular, were detected only sporadically, each in less than 1% of cows and on 8.6 and 10.5% of farms, respectively. Because of these small numbers of cases, interpretation of the associated risk factors should be made with caution.

Due to the funding restrictions and the previously unknown prevalence situation (of the diseases from publication I), no formal sample size calculation was performed. Nevertheless, a high number of study cows of more than 6000 cows from more than 150 farms was achieved. As far as is known, such a large and comprehensive study on udder health has not yet been conducted in Germany, which is why this work provides new and useful insights into the subject.

Another advantage of this study resulted from the selection of the sample. Especially for publication II on mastitis pathogens, the literature research revealed differences to other studies. Many studies on mastitis pathogen prevalence in Germany were conducted by diagnostic laboratories (Schwarz et al., 2010; Sorge, 2021). In most cases, the samples are a mixture of individual submissions (often diseased cows with mastitis) or samples from herd investigations, often from farms with an existing herd problem. Other studies focused on examinations of only clinically healthy cows (Tenhagen et al., 2006). The present work collected samples irrespective of the udder health status of the herd and cows and therefore provides a good overview of the udder health situation on Bavarian dairy farms.

2. Study region Bavaria and the generalizability for Germany

Bavaria is home to nearly one-third (28%) of Germany's dairy cows and almost half of all German dairy farms (46%; Statistisches Bundesamt, 2020).

This represents a large fraction of the German dairy livestock population overall. The farm structure of Bavarian dairy farms differs in some respects considerably from that of other German states. The herd size finds itself below the national average (Statistisches Bundesamt, 2020). This fact is partly related to the high number of tiestalls (39.3% of all farms tiestall in 2019, Schäffer et al., 2019), even though they are considerably declining (58.4% tiestalls in 2011).

Another major difference is the predominant breed in Bavaria. While Holstein cows are predominantly represented in most of the German states (Statistisches Bundesamt, 2020), Simmental is the most prevalent dairy breed in the Southern German states of Baden-Württemberg and Bavaria. In Bavaria, the Holsteins are only in third place (10.9%), after Simmental (77%) and Braunvieh (11.6%; Schäffer et al., 2019).

Since Simmental is a dual-purpose breed (compared to the dairy breed of Holstein-Frisian), milk yield in Bavaria (8045 kg/cow/year) is below the national average (8907 kg/cow/year; Schäffer et al., 2019).

Due to these nationwide differences, this work does provide results for one of the most dairy farming harbouring states, yet it does not necessarily apply to the rest of other German states.

3. Prevalence data

The present work is based on a cross-sectional study. A cross-sectional study provides a snapshot of how often a disease occurs at a certain point in time (Dohoo et al., 2009). In our case, it provides a major source of information on udder health in Bavarian dairy farms. Nevertheless, even after a careful random sampling, there are some points to keep in mind when evaluating the results.

First, some diseases have a longer duration than others. Diseases with a longer course have a higher chance of being diagnosed in a prevalence study than diseases that last a short time. For example, coli mastitis often has an acute course and a shorter duration than mastitis caused by other environmentally associated pathogens, e.g., streptococci (Smith and Hogan, 1993).

Second, some diseases affect only a certain period of time in the life of a study subject. As mentioned earlier, this is particularly true for UE (and thus UTD). Among other things, due to the increased pressure of the calf in the pelvic region cow, blood and lymph flow are reduced and consequently result in fluid accumulation in the udder (Okkema and Grandin, 2021). The temporal occurrence around parturition suggests an underrepresentation of the disease in the case of a cross-sectional study.

Thirdly, only lactating cows were studied in the present work. To follow up on the fact that UE only occurs around parturition, which may lead to the said underrepresentation of the disease, there is also the fact that (dry) cows are often kept in separate areas before calving, and not with the lactating cows, which in turn may further bias the results.

Moreover, the cows were examined and sampled during milking in the study. More severely diseased cows that were kept in extra cubicles, or cows excluded from milk due to food safety related aspects, may have thereby not been considered.

4. Risk factors

The fact that cross-sectional studies such as this one present a snapshot of the situation means that the evaluation of risk factors must always be interpreted in a differentiated way.

Cause and effect are not clearly assignable in prevalence studies, it remains open whether first a management practice was there and then the disease occurred or first the disease was there and subsequently the management practice was implemented. Therefore, one does not speak of an effect that a management practice or a cow factor has on the occurrence of a disease, but of an association.

Herd visits in this study were primarily made in the spring (73% of visits were in March and April). Furthermore, because not one visit was made in mid-summer (July and August), the influence of seasonality was not considered in the analyses. The timing of herd visits was due to factors including the expected unavailability of farmers at peak harvest times in mid-summer. To date, no seasonal effect has been found for UTD or UCD. For UE, Dentine and McDaniel (1983) also found no relationship between season and occurrence, whereas Melendez et al. (2006) found an increased risk of UE for primiparous cows in winter. Similarly, there appear to be seasonal differences in occurrence for some mastitis pathogens, primarily distinguishing between housing and pasture seasons (Duse et al., 2021).

When considering the results, they should always be critically evaluated and not universally applied to farms. Rather, the risk factors found serve as a guide and their association should be considered on an individual basis when the corresponding condition occurs on a farm. For example, in publication I, comfort rubber mats were identified as a risk factor for the occurrence of UCD in the herd. Ekman et al. (2018) also obtained a similar result. She suggested the increased accumulation of faeces due to the increased compressibility of these padded rubber mats led to a damage in the skin barrier and subsequently to UCD.

However, the conclusion from this should not be that comfort rubber mats should no longer be used. Comfort rubber mats have the advantage of being deformable to conform to the cow's body and reduce abnormalities such as hock lesions (Livesey et al., 2002). In addition, Herlin (1997) showed a preference of cows for comfort rubber mats, which is an advantage in terms of animal welfare. Instead, additional bedding to bind manure and urine, as well as cubicle hygiene, should be applied in case of increased occurrence of the disease.

Similarly, in publication II, the omission of blanket dry cow therapy was found to be a risk factor for *Str. dysgalactiae* in the herd. Even though antimicrobial resistance currently plays a minor role in mastitis pathogens only (Jong et al., 2018), with regard to the global antimicrobial resistance problem (World Health Organization, WHO, 2014), the recommendation to blanket dry cow therapy should be avoided.

In Germany, according to the “Tierarzneimittelgesetz” (TAMG), the Veterinary Medicinal Products Act, medication may only be used according to its indication, and according to the Regulation (EC) 2019/6 (European Comisson, 2018) on veterinary medicinal products, the prophylactic use of antibiotics in groups of animals is only permitted in exceptional cases. Antibiotic dry cow tubes should only be used selectively, or for metaphylaxis, i.e., for example in the case of known infection in the herd during an active control program.

5. Conclusion and perspective

With the help of this study, the apparent prevalence of teat warts, UE, UTD and UCD, as well as the different mastitis pathogens could be presented. It was found that teat warts, UE, UTD and UCD occurred only to a moderate level, often as single cases in a herd.

Mastitis pathogens, on the other hand, were detected in more than a quarter of the cows and almost a third of the cows showed subclinical or clinical mastitis.

Even though there are hardly any comparative data on the prevalences for udder diseases from publication I, these results can be considered positive for Bavarian udder health. In contrast, mastitis continues to be a common problem on Bavarian dairy farms and must continue to be actively controlled.

Also, some management practices could be found through this study, which indicate an association with the presence of the diseases, and mastitis pathogens, respectively.

More than once, free stall housing, or larger farms, were associated with the prevalence of UCD, or the prevalence of mastitis pathogens (*Str. uberis*, CNS). These farms should pay special attention to these diseases, and pathogens, respectively.

Furthermore, management practices that, when implemented, decreased the chances of mastitis pathogen occurrence were rarely implemented in the study herds. Thus, herds with *S. aureus* problems should consider post-milking teat disinfection and herds with *Str. dysgalactiae* should consider using internal teat sealants.

The present work can contribute to raise awareness of the mentioned conditions. Further studies should be conducted focusing on cows around parturition to assess the occurrence of primarily periparturient diseases such as UE or UTD. Also, when examining for udder diseases other than mastitis, dry cows as well as sick and therefore isolated cows should be examined along with the lactating herd. In addition, a year-round study period should be chosen to minimize seasonal influences. This could help getting a better overview of the prevalence of those diseases.

In general, this study provides a good basis for future comparable (prevalence) studies. For early detection of changes in disease occurrence as well as mastitis pathogen patterns over time, subsequent studies should be performed regularly. Further studies should be conducted to identify additional risk factors.

V. SUMMARY

Udder diseases represent an important issue for the dairy industry due to their impact on animal welfare, food safety, but also their financial consequences for dairy farms. The aim of this work was to determine the prevalence of teat warts, udder edema (UE), udder thigh dermatitis (UTD), and udder cleft dermatitis (UCD) and risk factors associated with these diseases at both herd- and cow-level within a cross-sectional study. Furthermore, the objective was to determine the prevalences of mastitis pathogens and to identify risk factors at the herd-level for the four most common pathogens.

For this purpose, over 6000 lactating cows from 152 Bavarian dairy farms were examined and sampled. In addition to the examination for the above-mentioned diseases, a California mastitis test was performed on each cow and quarter milk samples were taken. In addition, various hygiene scores (e.g., udder hygiene, leg hygiene) were recorded. Management practices and herd parameters were documented in a standardized checklist and cow data (lactation number, milk yield, etc.) were provided if available.

After statistical analysis of the data, teat warts, UE, UTD, and UCD were found to occur rarely, with teat warts in first place (4% of cows affected). Conventional milking at herd-level and cow somatic cell counts at cow-level were associated with the occurrence of teat warts. Poor depth of bedding material at herd-level and early lactation (day 0-60) at cow-level were associated with UE. For UCD, free stall housing and comfort rubber mats were identified as risk factors at the herd-level. Increased odds for UTD were identified for cows in first lactation compared to cows in later lactations.

Analysis of the prevalence of mastitis pathogens revealed that the most common pathogens isolated from all samples were coagulase-negative staphylococci (CNS, 4.4%), *Staphylococcus* (*S.*) *aureus* (2.9%), *Streptococcus* (*Str.*) *dysgalactiae* (0.9%), and *Str. uberis* (0.9%). The odds of CNS and *Str. uberis* were higher in freestalls than in tiestalls. Also, CNS and *Str. uberis* were isolated more frequently in large herds than in small herds. In herds with post-milking teat disinfection, the average intraherd prevalence of *S. aureus* was clearly lower than the intraherd prevalence of herds without post-milking teat disinfection. Herds that used internal

teat sealants and herds with blanket dry cow therapy had reduced odds of Str. *dysgalactiae* in the herd.

Overall, the udder diseases teat warts, UE, UTD, and UCD occurred fairly rarely. In contrast, about 32% of cows with at least one quarter were affected by mastitis. Therefore, despite the large number of studies on mastitis and many control measures, mastitis continues to be the most relevant udder disease. The most frequently isolated pathogens were CNS.

This work was able to determine the prevalence of teat warts, UE, UTD, UCD, and mastitis (or mastitis pathogens) on Bavarian dairy farms and risk factors associated with these conditions. Since UE and UTD in particular occur predominantly around the time of calving, further studies of each condition should be conducted to more precisely evaluate the problem. Further studies to determine the prevalences, especially of the mastitis pathogens, should be carried out regularly in the coming years in order to detect trends at an early stage and to be able to implement appropriate measures.

VI. ZUSAMMENFASSUNG

Eutererkrankungen spielen eine bedeutende Rolle für die Milchviehindustrie aufgrund ihres Einflusses auf das Tierwohl, die Lebensmittelsicherheit aber auch ihrer finanziellen Konsequenzen für die Milchviehbetriebe. Ziel dieser Arbeit war es im Rahmen einer Querschnittsstudie die Prävalenzen für Zitzenwarzen, Euterödeme, Zwischenschenkelekzeme und Eutergeschwüre sowie Risikofaktoren, die mit diesen Erkrankungen assoziiert sind, sowohl auf Herden- als auch auf Kuhebene zu bestimmen. Des Weiteren war es das Ziel die Prävalenzen von Mastitiserregern zu ermitteln und für die vier häufigsten Erreger Risikofaktoren auf Herdenebene zu identifizieren.

Hierfür wurden über 6000 laktierende Kühe von 152 bayerischen Milchviehbetrieben untersucht und beprobt. Neben der Untersuchung auf die oben genannten Erkrankungen, wurden bei jeder Kuh ein Schalmtest durchgeführt und Viertelgemelksproben genommen. Zusätzlich wurden diverse Hygiene Scores (u.a., Euterhygiene, Gliedmaßenhygiene) erfasst. Managementpraktiken und Herdenparameter wurden in einer standardisierten Checkliste festgehalten und Kuhdaten (Laktationsnummer, Milchleistung, etc.) sofern verfügbar bereitgestellt.

Nach statistischer Auswertung der Daten, zeigte sich, dass die Erkrankungen Zitzenwarzen, Euterödeme, Zwischenschenkelekzeme, und Eutergeschwüre nur selten auftraten, mit Zitzenwarzen an erster Stelle (4% der Kühe betroffen). Mit dem Auftreten von Zitzenwarzen wurden konventionelles Melken auf Herden- und Kuh-Zellzahl auf Kuh-Ebene assoziiert. Geringe Einstreutiefe (<5cm) auf Herdenebene und Frühlaktation (Tag 0-60) auf Kuhebene wurden mit Euterödem assoziiert. Für Eutergeschwür wurden auf Herdenebene die Laufstallhaltung und Komfort-Gummimatten als Risikofaktoren identifiziert. Erhöhte Chancen für Zwischenschenkelekzem konnten für Kühe in der Erstlaktation verglichen mit Kühen in späteren Laktationen festgestellt werden.

Bei der Bestimmung der Prävalenzen der Mastitiserreger zeigte sich, dass die am häufigsten aus allen Proben isolierten Erreger Koagulase-negative Staphylokokken (KNS) (4.4%), *Staphylococcus* (*S.*) *aureus* (2.9%), *Streptococcus* (*Str.*) *dysgalactiae* (0.9%), und *Str. uberis* (0.9%) waren. Die Chancen für KNS und *Str. uberis* waren in Laufstallhaltung höher als in Anbindehaltung. Auch wurden KNS und *Str. uberis* häufiger in großen Herden als in kleinen Herden isoliert. Bei Herden mit Zitzendesinfektion nach dem Melken lag die durchschnittliche Intraherden-Prävalenz von *S. aureus* klar unter der Intraherden-Prävalenz von Herden ohne Zitzendesinfektion nach dem Melken. Herden, die interne Zitzenversiegler nutzten und Herden mit generellem Trockenstellen hatten niedrigere Chancen für *Str. dysgalactiae* in der Herde.

Insgesamt waren die Eutererkrankungen Zitzenwarzen, Euterödeme, Zwischenschenkelekzeme, und Eutergeschwüre relativ selten vertreten. Hingegen waren etwa 32% der Kühe mit mindestens einem Viertel von Mastitis betroffen. Trotz einer Vielzahl an Studien zu Mastitis und vielen Bekämpfungsmaßnahmen stellt Mastitis daher weiterhin die bedeutsamste Eutererkrankung dar. Die am häufigsten isolierten Erreger waren die KNS.

Diese Arbeit konnte das Vorkommen von Zitzenwarzen, Euterödeme, Zwischenschenkelekzeme, und Eutergeschwüre und Mastitis (bzw. Mastitiserregern) auf bayerischen Milchviehbetrieben und Risikofaktoren, die mit diesen Konditionen assoziiert sind, ermitteln. Da besonders Euterödeme und Zwischenschenkelekzeme überwiegend um die Zeit der Abkalbung bei Kühen auftritt, sollten zur genaueren Ermittlung der Problematik weitere Studien zu den einzelnen Erkrankungen vorgenommen werden. Weitere Studien zur Ermittlung der Prävalenzen, insbesondere der Mastitiserreger, sollten in den kommenden Jahren regelmäßig durchgeführt werden, um frühzeitig Entwicklungen zu erkennen und entsprechend Maßnahmen ergreifen zu können.

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