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

Lucie J. Groh[1, 2], Rolf Mansfeld[2], Christian Baumgartner[3], and Ulrike S. Sorge[2]*

[1] *Tiergesundheitsdienst Bayern e.V. (Bavarian Animal Health Services), 85586 Poing, Germany*
[2] *Clinic for Ruminants with Ambulatory and Herd Health Services, Ludwig-Maximilian-University Munich (LMU Munich), 85764 Oberschleißheim, Germany*
[3] *Milchprüfring Bayern e.V. (Bavarian Association for Raw Milk Testing), 85283 Wolnzach, Germany*

**Corresponding author: Email: grohlucie@googlemail.com*

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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

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 $\alpha = 0.05$. 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*

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.

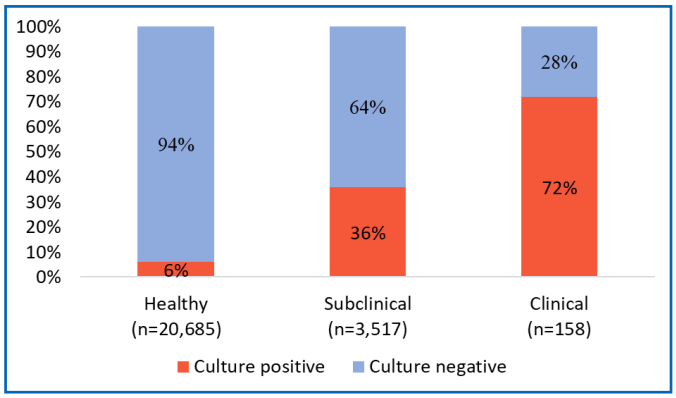

Figure 1. Proportion of aseptic quarter milk samples of 6,188 Bavarian study cows with pathogen detection by clinical status of the quarter. Healthy was defined here as samples from quarters with a negative California mastitis test (CMT) and without visual milk changes.

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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