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Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition

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Prevalence of mastitis pathogens in milk samples from dairy cows and heifers was studied over a period of 1 year (Aug 2005–Aug 2006) in ten dairy herds in Germany. Milk samples (n=8240) were collected from heifers without clinical mastitis at parturition (n=6915), from primiparous cows with clinical mastitis (n=751) and from older cows with clinical mastitis (n=574). Coagulase negative staphylococci (CNS) were the predominant group of bacteria isolated (46·8% of samples) from clinically healthy quarters of primiparous cows around parturition, followed by streptococci (12·6%), coliforms (4·7%) and *Staphylococcus aureus* (4·0%). Thirty-three percent of samples were negative on culture (range on farm level, 12·0–46·4%). In cases of clinical mastitis in primiparous and older cows, streptococci were the predominant finding (32·1 and 39·2%) followed by CNS (27·4 and 16·4%), coliforms (10·3 and 13·1%) and *Staph. aureus* (10·0 and 11·7%). Negative results were obtained from 21·3% (range, 0·0–30·6%) and 19·5% (range, 0·0–32·6%) of these samples. Results indicated substantial differences in the prevalence of pathogens among herds. There was a positive within-herd correlation between the monthly prevalences for *Streptococcus dysgalactiae* between the three groups of samples. This correlation was also found between clinical samples of primiparous and older cows for *Staph. aureus*. These correlations were not found for the other pathogens. Besides herd, prevalence of pathogens was influenced by parity, type of sample and season.

Keywords: Mastitis, streptococci, staphylococci, coliforms, *Staphylococcus aureus*, *Streptococcus dysgalactiae*.

Mastitis in primiparous cows is a permanent concern of dairy farmers worldwide. A lot of research has been conducted on risk factors for clinical mastitis and intramammary infection (IMI) at first parturition in primiparous cows (Waage et al. 1998; Waage et al. 2001; De Vliegher et al. 2003; Parker et al. 2007b). Extensive literature on the prevalence of pathogens in heifer secretions at parturition has been published (Oliver et al. 1997; Aarestrup & Jensen, 1997; Edinger et al. 1999; Borm et al. 2006; Oliver et al. 2007; Parker et al. 2007a). Likewise, a number of papers have reported on the pathogens associated with clinical mastitis in dairy heifers and primiparous cows (Waage et al. 1999; Tenhagen et al. 2001; Kalmus et al. 2006; Compton et al. 2007). Few papers have reported on the relationship of IMI at parturition and the risk of clinical mastitis during early lactation (Edinger et al. 1999; Parker et al. 2007a). The relationship of IMI, clinical mastitis and the associated pathogens in primiparous and older cows within the same herd has not been studied intensively.

Some studies have reported such a relationship without reference to the pathogens involved (Waage et al. 1998; Parker et al. 2007b). Data indicating an increased risk of mastitis in heifers housed together with older herdmates have been published for Canada (Bassel et al. 2003). The purpose of this study was to compare the prevalence of different bacteria in IMI in heifers at calving and in clinical mastitis in primiparous and older cows.

Material and Methods

The study was conducted on 10 commercial dairy farms in Brandenburg and Sachsen-Anhalt, Germany. The herds had on average 700 cows with an annual milk quota of 6200 t. Herd average annual milk production per cow was 7800–10 500 kg. All herds used their own replacement stock exclusively. Details of the herds are given in Table 1.

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Table 1. Characteristics of the ten study herds

Farm no	Lactating cows, *n*	Milk quota, $\times 10^{-6}$ kg	Production per cow, kg/year	No. of samples	Housing			Milking parlour
					Close-up period	During parturition	Primiparous cows in lactating herd	
1	723	7·0	7800	1047	Cubicles slatted floor	Two cows per pen on straw	Cubicles slatted floor	Rotary
2	295	3·2	8405	749	Deep straw litter	Group on straw	Cubicles plain floor	Heringbone
3	260	2·0	8704	482	Deep straw litter	Group on straw	Cubicles slatted floor	Heringbone
4	355	3·1	10 464	521	Deep straw litter	Group on straw	Cubicles plain floor	Heringbone
5	480	4·0	9853	1082	Deep straw litter	Single on straw	Deep straw litter	Parallel
6	1173	10·9	8893	1501	Cubicles slatted floor	Group on straw	Cubicles slatted floor	Parallel
7	868	8·5	9588	1011	Deep straw litter	Group on straw	Cubicles slatted floor	Rotary
8	900	6·8	9363	843	Cubicles slatted floor	Single on straw	Cubicles plain floor	Rotary
9	1200	10·0	8685	1242	Deep straw litter	Single on straw	Cubicles slatted floor	Rotary
10	750	6·4	9259	571	Tie stall	Tie stall	Cubicles slatted floor	Parallel

Collection of milk samples

Milk samples were collected between August 2005 and August 2006. Farmers were advised to sample any case of clinical mastitis during the first month of lactation prior to treatment and a maximum of five heifers per week within 48 h of parturition. Colostrum samples were chosen to minimize the risk that primiparous cows acquired IMI post partum either during the milking process or from the environment. Farmers were advised not to pre-select heifers based on their health, but to sample the first five heifers. The upper limit was voluntarily chosen to limit the total number of samples for financial reasons.

Two types of samples were differentiated: 'clinical samples' were from quarters with clinical mastitis. Clinical mastitis was diagnosed by the farm workers. The diagnosis was mainly based on visible changes of the secretion and/or the consistency of the mammary tissue. These samples were further characterized by the age of the cows (primiparous *v.* multiparous) and the time relative to parturition when they were collected. 'Non-clinical samples' were colostrum samples from clinically healthy quarters of primiparous cows.

Samples were collected by trained farm personnel. Workers were trained by explanation and demonstration of the correct procedure by one of the investigators. After cleaning of the teats and discarding the first streaks of milk, teats were wiped with commercial towels for teat disinfection as provided together with intramammary drugs by the pharmaceutical companies. Products of various companies were used. Samples were collected in sterile vials and stored in a refrigerator at approx. 4 °C until weekly transportation to the laboratory and analysis.

Compliance with the sampling protocol varied between farms and workers on farms. As cows that were to be included in the study and cows that were not to be included in the study anymore were often housed together, the farm workers were not always aware of the actual days in milk for the respective cows. To avoid missing too many cows, we chose to present data from all the samples but stratified according to the stage of lactation.

Laboratory analysis

In the laboratory 0·01 ml of milk was streaked out on one half of an agar dish (Blood Agar Base No. 2, Oxoid, Wesel, supplemented with 5% sheep blood and 0·1% aesculin). After 48 h of incubation growth was evaluated and preliminary identification by colony morphology and haemolysis was carried out.

Staphylococcus aureus was differentiated from coagulase negative staphylococci (CNS) using a commercial tube coagulase test (BBL Coagulase Plasma, Rabbit; Becton, Dickinson and Company, Heidelberg, Germany). Streptococci were differentiated using the CAMP Test and a commercial test to define Lancefield groups (Streptococcal grouping kit; Oxoid, Wesel, Germany). Streptococci were differentiated into *Streptococcus agalactiae* (positive CAMP test and Lancefield group B), *Str. dysgalactiae* (aesculin-negative, Lancefield C), *Str. uberis* (aesculin-positive, no growth on salt, non Lancefield D) and other streptococci.

Coliforms, yeasts and *Arcanobacterium pyogenes* were identified by colony morphology and Gram-staining. All other bacteria were summarized as 'others' for the purpose of this study. Samples with growth of two pathogens were regarded as positive for both pathogens. Samples with growth of more than two pathogens were classified as contaminated and withdrawn from the analysis. For *Staph. aureus* and *Arc. pyogenes* single colonies (i.e. ~100 cfu/ml) were defined as positive. For all other pathogens we defined a minimum of three colonies (i.e. ~300 cfu/ml) as threshold value for a sample to be regarded as positive.

Statistical analysis

Analysis was based on quarters. Contaminated samples were withdrawn from the analysis. Prevalences of pathogens in the text and tables are given as number of samples positive for a specific pathogen or group of pathogens, divided by the number of samples that could be analysed in the respective category. Unless stated otherwise,

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Table 2. Prevalence of pathogens in milk samples from quarters without clinical mastitis of primiparous cows around parturition (n=6915)†

Parameter	Days relative to parturition			Overall %‡ (herd range %)
	−6 to −1	0 to 2	3 to 7	
Quarters samples analysed, *n*	38	6510	367	6915
Negative % (*n*)	21·1 (8)	32·8 (2133)	39·2 (144)	33·0 (12·0–46·4)
Coagulase-negative staphylococci % (*n*)	52·6 (20)	47·3 (3076)	39·5 (145)	46·9 (38·7–55·8)
Staphylococcus aureus % (*n*)	7·9 (3)	3·9 (253)	4·9 (18)	4·0 (0·8–20·9)
Coliforms % (*n*)	7·9 (3)	4·8 (308)	3·0 (11)	4·7 (1·1–8·8)
Streptococcus spp. § % (*n*)	5·3 (2)	7·1 (460)	6·8 (25)	7·0 (2·9–11·8)
Streptococcus agalactiae % (*n*)	0·0 (0)	0·1 (7)	0·3 (1)	0·1 (0–0·8)
Streptococcus dysgalactiae % (*n*)	0·0 (0)	3·2 (209)	2·5 (9)	3·2 (0·8–8·5)
Streptococcus uberis % (*n*)	0·0 (0)	2·4 (158)	0·3 (1)	2·3 (0·6–9·5)
Arcanobacterium pyogenes % (*n*)	0·0 (0)	0·9 (61)	3·3 (12)	1·1 (0–5·1)
Yeast % (*n*)	0·0 (0)	0·0 (0)	0·3 (1)	0·0 (0–0·1)
Others % (*n*)	15·8 (6)	9·9 (644)	13·6 (50)	10·1 (2·6–15·0)

† Samples do not add up to 100% because of mixed infections. Proportions are given as number of samples positive for a given pathogen divided by number of samples analysed over the complete study period, given in percent
‡ Calculation based on samples
§ Excluding *Str. agalactiae, Str. dysgalactiae* and *Str. uberis*

prevalence was calculated for the complete study period. Values are given in percent.

Factors influencing the outcome of the milk sample were studied using separate binary logistic regression for the six most prevalent pathogens. The outcome variable was presence of the respective pathogen (0=not present 1=present). The independent factors were herd (10 categories), season (0=summer, April–September, *v.* 1=winter, October–March), location of quarter (fore *v.* hindquarter), type of sample (0=colostrum *v.* 1=clinical mastitis) and parity (0=primiparous *v.* 1=multiparous). As location of quarter had no significant effect, it was dropped from the final model.

The relationship between the prevalence of pathogens in the three groups of samples (primiparous colostrum, primiparous clinical mastitis, multiparous clinical mastitis) was analysed using Spearmans correlation coefficient. Monthly prevalences were calculated as the number of positive samples divided by the total number of samples of this type in the herd and month. The correlation was calculated for the monthly values, i.e. 12 values per herd per category and pathogen. All analyses were carried out using SPSS Version 12.0 (SPSS Inc. München, Germany).

Results

Overall, 7617 non-clinical samples from heifers were collected. Of those 702 (9·2%) were contaminated or broken and could not be analysed. Of the 811 clinical samples from primiparous cows, 60 (7·4%) were contaminated and excluded from the analysis. Forty-seven of the 621 clinical mastitis samples from older cows (7·6%) were also excluded from the analysis because of contamination.

Factors associated with the prevalence of specific pathogens in the sample

Prevalence of pathogens in the different types of milk samples is presented in Tables 2–4. Table 5 presents the summary results of the logistic regression on factors associated with the presence of a pathogen in a sample. Herd had a significant impact on the prevalence of all mastitis pathogens (Table 5).

Overall, CNS were most often isolated. Their proportion was highest in samples of clinically healthy quarters of heifers prior to and within 48 h after parturition. Samples collected later and samples from cases of clinical mastitis in cows and heifers contained CNS less often. Furthermore,

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Table 3. Prevalence of pathogens in samples from cases of clinical mastitis in primiparous cows (n=751)†

	Days after parturition				Overall %‡ (herd range %)
	0–2	3–7	8–30	31–305	
Quarter milk samples analysed, *n*	506	60	94	91	751
Negative % (*n*)	20·6 (104)	11·6 (7)	33·0 (31)	19·8 (18)	21·3 (0–30·6)
Coagulase-negative staphylococci % (*n*)	33·2 (168)	31·6 (19)	10·6 (10)	9·9 (9)	27·4 (11·1–40·3)
Staphylococcus aureus % (*n*)	10·9 (55)	8·3 (5)	8·5 (8)	7·7 (7)	10·0 (0–22·6)
Coliforms % (*n*)	8·7 (44)	6·7 (4)	8·5 (8)	23·1 (21)	10·3 (5·2–25·0)
Streptococcus spp. § % (*n*)	9·3 (47)	10·0 (6)	14·9 (14)	5·5 (5)	9·6 (5·3–26·7)
Streptococcus agalactiae % (*n*)	0·6 (3)	0·0 (0)	0·0 (0)	0·0 (0)	0·1 (0–2·6)
Streptococcus dysgalactiae % (*n*)	16·0 (81)	16·6 (10)	4·3 (4)	7·7 (7)	13·6 (0–26·0)
Streptococcus uberis % (*n*)	6·1 (31)	6·7 (4)	14·9 (14)	16·5 (15)	8·5 (2·9–50·0)
Arcanobacterium pyogenes % (*n*)	5·7 (29)	16·6 (10)	3·2 (3)	1·1 (1)	5·7 (0–9·4)
Yeast % (*n*)	0·0 (0)	0·0 (0)	4·3 (4)	4·4 (4)	1·1 (0–9·7)
Others % (*n*)	8·9 (45)	6·6 (4)	11·7 (11)	8·8 (8)	9·1 (0–17·9)

† Samples do not add up to 100% because of mixed infections. Proportions are given as number of samples positive for a given pathogen divided by number of samples analysed over the complete study period, given in percent
‡ Calculation based on samples
§ Excluding *Str. agalactiae, Str. dysgalactiae* and *Str. uberis*

the prevalence of CNS was slightly lower in winter than in summer (45 *v.* 49% and 25 *v.* 30% in primiparous cows without and with mastitis, respectively, 16 *v.* 17% in cows) (Table 5).

Staph. aureus was identified more often from clinical mastitis samples than from non-clinical samples. The higher prevalence in clinical mastitis samples from older cows compared with clinical samples from primiparous cows was not significant (OR 1·43, 95% CI, 0·98–2·10). With respect to season, data were not conclusive. Prevalence of *Staph. aureus* was higher in clinical samples of older cows in winter than in summer. In non-clinical samples and clinical samples from primiparous cows the prevalence was higher in summer.

Besides CNS, streptococci were the group of bacteria with the highest overall prevalence. *Str. agalactiae* was only found occasionally (5 isolates from multiparous cows, 11 from primiparous cows). All other streptococci, i.e. *Str. dysgalactiae, Str. uberis* and other streptococci were more prevalent in clinical mastitis than in non-clinical samples and were more frequent in winter than in summer. Contrary to the other streptococci, *Str. dysgalactiae* was more often isolated from primiparous than from older cows.

Coliforms were 2·7-times more likely to be detected in clinical than in non-clinical samples (Table 5) and were isolated more often in summer. The higher prevalence in older cows was not significant (OR 1·39, 95% CI, 0·97–2·00).

Correlation of the prevalence of specific pathogens on herd level

Only a few significant correlations were detected (Table 6). Prevalence of *Staph. aureus* in clinical mastitis samples from older cows was positively correlated ($P<0·01$) with the prevalence of *Staph. aureus* in clinical mastitis samples from primiparous cows. However, both were not related to the prevalence in non-clinical colostral samples.

Prevalence of *Str. dysgalactiae* in clinical mastitis samples was also correlated among the different age groups. For *Str. dysgalactiae* there was also a significant positive correlation of the prevalence in non-clinical samples with the prevalence in clinical mastitis samples from primiparous and multiparous cows.

All other prevalences were not significantly correlated among the three groups of samples.

Two samples from the same quarter (i.e. one non-clinical colostral sample and a clinical sample collected later) were available in 171 cases. Because of contamination of one of the samples, 29 pairs (17%) could not be analysed. If the non-clinical samples contained major pathogens

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Table 4. Prevalence of pathogens in samples from cases of clinical mastitis in multiparous cows (n=574)†

Time of sampling relative to parturition	Day −6 to −1	Day 0 to 2	Day 3 to 7	Day 8 to 30	Day 31 to 305	Overall %‡ (herd range %)
Quarter milk samples analysed, *n*	5	85	41	157	286	574
Negative % (*n*)	20·0 (1)	17·6 (15)	12·2 (5)	18·5 (29)	21·7 (62)	19·5 (0–32·6)
Coagulase-negative staphylotococci % (*n*)	40·0 (2)	20·0 (17)	14·6 (6)	14·7 (23)	16·1 (46)	16·4 (4·0–23·8)
Staphylococcus aureus % (*n*)	20·0 (1)	14·1 (12)	24·4 (10)	5·7 (9)	12·2 (35)	11·7 (0–28·8)
Coliforms % (*n*)	0·0 (0)	5·9 (5)	12·2 (5)	17·2 (27)	13·3 (38)	13·1 (7·7–42·9)
Streptococcus spp. § % (*n*)	20·0 (1)	18·8 (16)	7·3 (3)	17·8 (28)	15·0 (43)	15·9 (11·0–25·4)
Streptococcus agalactiae % (*n*)	0·0 (0)	5·9 (5)	0·0 (0)	0·0 (0)	0·0 (0)	0·9 (0–12·0)
Streptococcus dysgalactiae % (*n*)	20·0 (1)	10·6 (9)	12·2 (5)	0·6 (1)	6·6 (19)	6·1 (0–40·0)
Streptococcus uberis % (*n*)	20·0 (1)	11·8 (10)	17·1 (7)	19·1 (30)	16·1 (46)	16·4 (1·8–30·0)
Arcanobacterium pyogenes % (*n*)	0·0 (0)	8·3 (6)	0·0 (0)	4·5 (7)	1·7 (5)	3·1 (0–10·5)
Yeast % (*n*)	0·0 (0)	0·0 (0)	0·0 (0)	2·6 (4)	0·4 (1)	0·9 (0–2·1)
Others % (*n*)	0·0 (0)	8·3 (6)	14·6 (6)	10·2 (16)	8·7 (25)	9·2 (0–18·6)

† Samples do not add up to 100% because of mixed infections. Proportions are given as number of samples positive for a given pathogen divided by number of samples analysed over the complete study period, given in percent
‡ Calculation based on samples
§ Excluding *Str. agalactiae, Str. dysgalactiae* and *Str. uberis*

(*Staph. aureus*, coliforms, streptococci) these were also detected in the clinical samples collected later in 66% of the cases (32/49). With CNS, this was only the case in 33% (16/48).

Discussion

CNS were the group of bacteria with the highest prevalence in non-clinical samples in all of the 10 herds studied. They are commonly classified as minor pathogens (IDF, 1999). With respect to udder health in primiparous cows they have received considerable attention (Aarestrup & Jensen, 1997; Edinger et al. 2000; Tenhagen et al. 2001; Borm et al. 2006; Tenhagen et al. 2006; Compton et al. 2007). It has been demonstrated that IMI with CNS at parturition was associated with higher cell counts and lower milk yield in the first lactation (Timms & Schultz, 1987). In our study their prevalence in samples from cases of clinical mastitis was lower than in the non-clinical samples. This is a substantial difference from all other pathogens included in this study. Likewise the proportion of quarters harbouring CNS at parturition that later developed clinical mastitis with the same group of pathogens was lower (33%) than could be observed for major pathogens (66%).

Statistically the lower prevalence in clinical mastitis samples suggests a protective effect of CNS against clinical mastitis at parturition. However, as CNS are an integral part of the normal skin flora, they might also have been derived from colonized streak canals or colonization of the teat cistern. Colonization of the streak canal could explain the substantial reduction of CNS in quarters sampled a couple of days into lactation that has been reported before (Aarestrup & Jensen, 1997; Edinger et al. 2000; Calvinho et al. 2007). Such a reduction was also reported for other pathogens (Compton et al. 2007). For this reason, it is difficult to compare the culture results of milk samples collected at different intervals after calving. Isolation of bacteria from colostral samples may be a sensitive detection method but may also include 'false positives' that show self cure during the first days post partum. On the other hand, clinical mastitis is a frequent event in early lactation and infection pressure in fresh cow groups is high because of shedding of milk and lochia into the environment. Therefore postponing sampling may result in a substantial shift of pathogens that are isolated, and consequently, the pathogen pattern may no longer reflect the situation around parturition.

CNS are a heterogenous group of bacteria. In this study, CNS were not identified to the species level. Therefore no information is available on whether the isolates from quarters with clinical mastitis differed from those with no clinical mastitis. Further investigations are warranted to

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Table 5. Summary results of logistic regression concerning the risk of the presence of a pathogen in a milk sample

Pathogen	Covariate	Coefficient of regression	SE	df	*P value*	Odds ratio	95% CI
Coagulase-negative staphylococci	Herd	—	—	9	0·000	—	—
	Parity	−0·732	0·143	1	0·000	0·481	0·363–0·636
	Type of sample	−0·779	0·091	1	0·000	0·459	0·384–0·549
	Season	−0·148	0·046	1	0·001	0·863	0·788–0·944
	Constant	1·936	0·159	1	0·000	6·934	—
Coliforms	Herd	—	—	9	0·000	—	—
	Parity	0·331	0·185	1	0·073	1·392	0·969–2·000
	Type of sample	0·997	0·151	1	0·000	2·711	2·015–3·646
	Season	−0·882	0·103	1	0·000	0·414	0·338–0·507
	Constant	−3·004	0·263	1	0·000	0·050	—
Staphylococcus aureus	Herd	—	—	9	0·000	—	—
	Parity	0·359	0·194	1	0·065	1·432	0·978–2·096
	Type of sample	0·768	0·155	1	0·000	2·156	1·590–2·923
	Season	−0·263	0·106	1	0·013	0·769	0·625–0·946
	Constant	−4·191	0·308	1	0·000	0·015	—
Streptococcus spp.†	Herd	—	—	9	0·000	—	—
	Parity	0·508	0·140	1	0·000	1·663	1·264–2·188
	Type of sample	0·967	0·117	1	0·000	2·629	2·089–3·309
	Season	0·536	0·073	1	0·000	1·709	1·481–1·973
	Constant	−4·365	0·202	1	0·000	0·013	—
Streptococcus dysgalactiae	Herd	—	—	9	0·000	—	—
	Parity	−0·554	0·215	1	0·010	0·575	0·377–0·876
	Type of sample	1·056	0·142	1	0·000	2·875	2·175–3·800
	Season	0·494	0·115	1	0·000	1·639	1·307–2·054
	Constant	−5·442	0·407	1	0·000	0·004	—
Streptococcus uberis	Herd	—	—	9	0·000	—	—
	Parity	0·288	0·187	1	0·124	1·334	0·924–1·925
	Type of sample	1·575	0·173	1	0·000	4·833	3·440–6·790
	Season	0·808	0·132	1	0·000	2·244	1·732–2·908
	Constant	−6·134	0·325	1	0·000	0·002	—

† Excluding *Str. dysgalactiae, Str. uberis* and *Str. agalactiae*

analyse whether there are differences between the various CNS that can be isolated from milk samples.

Prevalence of *Staph. aureus* in dairy herds is reported to be higher than that of most other major mastitis pathogens (Tenhagen et al. 2006; Østerås et al. 2006). Although it is characterized as a contagious pathogen that is predominantly spread during the milking process, it has also been isolated from heifers and primiparous cows prior to and at parturition (Roberson et al. 1998; Edinger et al. 2000). In the present study, prevalence of *Staph. aureus* was higher in mastitis samples than in non-clinical samples. While in primiparous cows it was found more often in summer than in winter, the opposite was observed for the older cows with clinical mastitis. In a recent survey from Norway, prevalence of *Staph. aureus* was higher in summer (Østerås et al. 2006). Prevalence of *Staph. aureus* in clinical mastitis samples from older cows did not differ significantly from those of primiparous cows. In contrast, we reported that prevalence of *Staph. aureus* in samples from clinically healthy cows was higher in older than in younger cows in our region (Tenhagen et al. 2006). However, this was observed in later lactation. In early lactation, the difference was not significant. This is in accordance with the data of this study that focused on the beginning of lactation. In a report from Norway the prevalence of *Staph. aureus* in primiparous and older cows did not differ significantly (Østerås et al. 2006).

Prevalence of *Staph. aureus* in clinical samples from primiparous and older cows was positively correlated. IMI with *Staph. aureus* is acquired at milking time especially if milking-time hygiene is inappropriate. Therefore the correlation between the mastitis cases in primiparous and older cows may reflect the spread of *Staph. aureus* from multiparous to primiparous cows and vice versa during milking. Prevalence of *Staph. aureus* in non-clinical samples, i.e. around parturition, was not correlated to the prevalence in clinical samples. Most of the non-clinical samples were collected prior to first-milking. Therefore infection during the milking process can be ruled out for the animals. The risk of being infected at parturition was therefore independent of the risk of getting infected during the milking process.

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Table 6. Herd-level rank correlations (Spearmans ρ) of the prevalences of selected pathogens per month† in non-clinical samples from primiparous cows around parturition, and from clinical samples of primiparous and older cows. Correlation is based on the monthly values

Pathogen		Clinical samples first lactation	Non-clinical samples first lactation
Coagulase-negative staphylococci	Clinical samples first lactation	—	0·427
	>first lactation	0·333	0·025
Staphylococcus aureus	Clinical samples first lactation	—	0·102
	>first lactation	0·915**	0·167
Coliforms	Clinical samples first lactation	—	0·218
	>first lactation	0·317	−0·393
Streptococcus spp. ‡	Clinical samples first lactation	—	0·454
	>first lactation	−0·283	−0·067
Streptococcus dysgalactiae	Clinical samples first lactation	—	0·667*
	>first lactation	0·949**	0·746*
Streptococcus uberis	Clinical samples first lactation	—	0·393
	>first lactation	0·233	0·159

* $P<0·05$; ** $P<0·01$

† Herd prevalence per month = number of positive samples/number of samples collected per herd and month

‡ Excluding *Str. dysgalactiae, Str. uberis* and *Str. agalactiae*

Non-agalactiae streptococci are one of the major groups of pathogens associated with mastitis. As a group, their prevalence in samples from cases of clinical mastitis in primiparous and in older cows was highest. Their prevalence in non-clinical samples was substantially lower. This is in line with their classification as major pathogens and with data from other studies (Edinger et al. 2000). The higher prevalence of environmental streptococci in older cows, compared with primiparous cows, is supported by recent data from large dairy herds in Germany (Tenhagen et al. 2006).

In the quarters that were sampled twice, at parturition and further into lactation in the case of clinical mastitis, 66% of the quarters harbouring streptococci at parturition also contained streptococci, when their first case of clinical mastitis was detected.

Str. dysgalactiae showed some differences from the other streptococci. The major difference was the strong positive correlation of the prevalence of *Str. dysgalactiae* in non-clinical samples from primiparous cows, clinical samples from primiparous cows and clinical samples from multiparous cows. *Str. dysgalactiae* was the only pathogen that showed these strong correlations between all three types of samples. The relationship between the clinical samples from primiparous and older cows may be a typical feature of a contagious pathogen because the feature was also observed in *Staph. aureus* but not observed in other streptococci and in coliforms.

For *Staph. aureus* no correlation between clinical and non-clinical samples was observed. It is possible that *Str. dysgalactiae* also has environmental reservoirs that are a non-permanent source of infection. Potential candidates for these reservoirs include flies. Another possible explanation is that heifers act as a reservoir for *Str. dysgalactiae* and that the pathogen is spread from heifers and primiparous cows to older cows during milking. In line with this explanation, *Str. dysgalactiae* was more prevalent in primiparous than in older cows. In line with both hypotheses, *Str. dysgalactiae* has been associated with cases of summer mastitis in heifers, a condition that is commonly attributed to *Arc. pyogenes* (Madsen et al. 1992). Flies play an essential role in the epidemiology of this condition.

Extraordinary features of *Str. dysgalactiae* especially regarding heifers, have been observed before. Strains of *Str. dysgalactiae* that were isolated from heifers in the week prior to parturition were observed in the same quarter after parturition (Aarestrup & Jensen, 1997). *Str. dysgalactiae* has been reported to invade epithelial cells and survive there for a longer period without damaging the cells (Calvinho & Oliver, 1998). This may explain the comparatively high proportion of positive colostral samples without clinical manifestations.

The higher prevalence of *Str. dysgalactiae* in primiparous than in older cows is in contrast to two other recently published studies (Østerås et al. 2006; Tenhagen et al. 2006). However, these studies dealt with non-clinical samples further into lactation, which were not investigated in this study.

Prevalence of *Str. dysgalactiae* in Scandinavian studies in heifers was always higher than that of other streptococci (Jonsson et al. 1991; Aarestrup & Jensen, 1997; Waage et al. 1999). In line with this observation, in epidemiological studies in Norway, *Str. dysgalactiae* was the second most isolated major mastitis pathogen. In North American studies, *Str. dysgalactiae* was observed less often and in many studies was not reported as a separate pathogen.

In contrast to the Norwegian investigation, in our study *Str. dysgalactiae* was more prevalent in winter than in summer, just like the other streptococci. In the Norwegian survey, *Str. dysgalactiae* and *Str. uberis* were more prevalent in summer than in winter. The reason for the difference is not clear. However, there are substantial differences in herd sizes and climatic conditions between the Norwegian herds (15 cows) and the herds that we included in the study (700 cows).

As expected, *Str. uberis* was far more prevalent in clinical than in non-clinical samples (Compton et al. 2007). Unlike in studies from North America (Smith et al. 1985; Todhunter et al. 1995) *Str. uberis* was more prevalent in winter than in summer (Table 5). Differences in the

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prevalence of *Str. uberis* between primiparous and older cows that have been observed in other studies were not prominent in our data.

Coliforms have been isolated from the majority of cases of clinical mastitis in several studies (Barker et al. 1998; Sargeant et al. 1998; Shpigel et al. 1998). In our study herds, coliforms were isolated from 10% of clinical mastitis samples from primiparous cows and from 13% of clinical mastitis samples from older cows. However, as with all pathogens, the contribution of coliforms varied substantially between herds ranging from 5 to 25% in primiparous and from 7 to 43% in older cows. As in other studies, coliforms were found more often in summer than in winter. In non-clinical samples the prevalence of coliforms was significantly lower.

The association of herd with the prevalence of the pathogens and the large variation in the prevalences between herds points to the potential benefit of an improved herd management for the mastitis situation in the herd. The herds showed some variability concerning housing, production level and management. However, no overt associations between single items and the prevalence of mastitis were observed and the limited number of herds did not allow for statistical analyses concerning the association of specific herd conditions and the prevalence of pathogens.

Conclusion

Our data indicate that clinical mastitis in primiparous and older cows differs with respect to pathogen pattern. Furthermore, pathogens isolated from clinically healthy quarters of primiparous cows at parturition differ from those isolated from clinical mastitis. The strong positive correlation of the prevalences of *Str. dysgalactiae* in all three types of samples calls for further investigation into the genetic variability of the strains. It should be analysed, whether the relationship is caused by clonal spread of strains or by increased infection pressure from the environment.

The differences between non-clinical and clinical samples indicate that reports on the reduction of peri-partum IMI in heifers by management have to differentiate between the various pathogens especially between major and minor pathogens.

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