Milk Culture Results in a Large Norwegian Survey—Effects of Season, Parity, Days in Milk, Resistance, and Clustering

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ABSTRACT

A nationwide random computerized assignment survey that included 3,538 sets of 4 quarter milk samples from 2,834 dairy cows was conducted during 2000. Every fifth cow from every 50th herd was randomly selected for sampling and culture during each quarter of the year. Milk culture results of pathogens known to be related to mastitis were recorded regardless of whether mastitis had been indicated by any inflammatory measure or not. Farmers were blinded to all test results to minimize any potential interventions that might be prompted by the results. The most prevalent isolate was *Staphylococcus aureus*, which was identified in 8.2% of the quarter milk samples. More than 15 colony-forming units/0.01 mL of *Staph. aureus* were found in 4.3% of the quarter milk samples, whereas 3.5% had only 1 to 3 colony-forming units/0.01 mL. *Streptococcus dysgalactiae*, coagulase-negative staphylococci (CNS), and *Streptococcus uberis* were isolated from 1.2, 3.3, and 0.4% of quarter milk samples, respectively. No isolates were found in 76.6% of the quarter milk samples tested. Among individual cows, 22.2% had an isolate of *Staph. aureus* in ≥ 1 quarter. Only *Strep. dysgalactiae* exhibited a higher prevalence with increased parity. Prevalence of *Staph. aureus* decreased throughout days in milk, but prevalence of *Strep. dysgalactiae* increased. There was a strong seasonal effect; the highest prevalence of *Strep. dysgalactiae* and CNS was observed during April and May (late indoor season), and the highest prevalence of *Staph. aureus* and *Strep. uberis* was observed during June and July (the outdoor season). A substantial within-cow clustering effect was found for *Strep. dysgalactiae*, *Staph. aureus*, and CNS. Additionally, a within-herd effect was found for *Strep. uberis*, penicillin-resistant *Staph. aureus*, total *Staph. aureus*, and CNS. No within-county cluster effect was found. Lastly, both *Staph. aureus* and CNS exhibited a surprisingly high seasonal effect regarding the prevalence of resis-

tance to penicillin G. Penicillin resistance of *Staph. aureus* was likely due to higher prevalence of *Staph. aureus* as a whole, but for CNS, there was also an additional increase caused by a higher proportional rate of penicillin resistance during the late indoor season.

Key words: parity, days in milk, season, penicillin resistance

INTRODUCTION

Norway's organized mastitis control program, which was established in 1982, has been an integral part of the Norwegian Cattle Health Services since 1995. The goal of the mastitis control program is to reduce the overall cost of udder health problems for the individual farm by pursuing a strategy that addresses current udder health problems. There are 2 main classes of mastitis. The first is clinical mastitis, which manifests signs observed from the animal or the milk. The other is subclinical mastitis, which produces no visible signs from the udder except when using diagnostic tools (International Dairy Federation, 1999). The distribution of mastitis pathogens may differ between clinical and subclinical mastitis, as clinical mastitis can be caused by pathogens that are present for a short duration and produce obvious clinical signs. Conversely, subclinical mastitis can be caused by pathogens that are present for longer periods of time and produce negligible clinical signs.

In recent years, the Norwegian dairy industry has experienced a marked decrease in bulk milk SCC (BMSCC) from 173,000 to 113,000/mL (geometric mean) from 1988 to 2000. In contrast, the incidence of clinical cases has increased from 0.30 primary cases per cow in 1998 to 0.35 primary cases per cow in 1994; then, a decrease was observed until 2000 when number of primary cases per cow fell to 0.23 (Norwegian Cattle Health Services, 2004). A minimal relationship was reported between clinical cases at herd level and BMSCC (Valde et al., 2005). The reason for this could be that low BMSCC levels can be achieved through targeted therapy of cows with high SCC, withholding high SCC milk either at quarter or cow level from delivery, or both. Usually routine samples sent to the mastitis labo-

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ratories for microbiological culture are from selected cows with a high cow milk SCC and from herds with high BMSCC. Such samples may not reflect the true prevalence of pathogens in the dairy population.

Subclinical mastitis is a common reservoir of infectious pathogens (Yancey et al., 1991). Information about the distribution and antibiotic susceptibility pattern of mastitis pathogens in milk samples in the dairy population is important for strategic decision making and optimal planning of mastitis control programs. A recently reported national surveillance study in Finland (Pitkälä et al., 2004) demonstrated a major shift in the pathogens identified from the predominance of *Staphylococcus aureus* in 1995 to CNS in 2001. Few countries have conducted national surveillance studies, and to date, no such study has been carried out on the Norwegian dairy population. Information concerning the prevalence of mastitis pathogens in relation to season, DIM, parity, and possible clustering effects within cow, herd, or county would be important in terms of developing a strategy for mastitis control, therapy, and design of therapeutic trials.

The goals of this epidemiological study were to conduct a random survey of udder quarters and cows in Norway to describe the point prevalence and distribution of possible subclinical mastitis pathogens, to describe the antimicrobial resistance patterns of these pathogens, and to describe possible associations between the presence of a pathogen and factors such as season, DIM, parity, and any cluster effects within cow, herd, or county.

MATERIALS AND METHODS

Sampling

All Norwegian dairy herds ($n = 20,858$) taking part in the Norwegian Dairy Herd Recording System (NDHRS) as of December 31, 1998 were eligible for inclusion in the survey. This consisted of 89.2% of all dairy herds in Norway. A list of these herds was sorted according to identification number, and after the first herd was determined by assigning a random starting number between 1 and 49, every 50th herd was selected to join the survey. Thus, a total of 417 Norwegian dairy herds were included at the start of the survey. The survey started on January 1, 2000, and ended on January 1, 2001.

A complete list was compiled including every cow within the selected herds that had ≥ 1 calving. These cows were sorted according to their unique identification number, and after the first cow was determined by assigning a random starting number from 1 to 4, every fifth cow was selected for sampling. The lists of cows to be sampled were compiled in January, March,

June, and September. These lists were sent to the dairy advisors, who collected the milk samples and shipped them by priority mail to the Dairy Association Mastitis Laboratory in Molde, Norway. Samples from each quarter from all selected cows were sampled once during the following 3 mo. The within-herd selection of cows was designed so that each cow would have an even chance of being selected within each 3-mo sampling period. If a cow selected had been culled at the time of sampling, it was replaced with the most recent first parity cow entering that dairy herd. The number of cows selected in each 3-mo period varied from 1,147 to 1,179.

Inclusions and Exclusions

All together, 67 of the 417 herds were excluded before or during the study. Fifteen (3.6%) of these 417 herds had discontinued their milk production during 1999, and 36 of the remaining 402 herds (9.0%) stopped their milk production during 2000. An additional 16 herds were excluded from the study because 1) the farmer refused to cooperate (7 of 16), 2) farmers combined their herds with other farmers to form cooperative farms (3 of 16), 3) farmers terminated their membership in the animal recording scheme (3 of 16), and 4) other reasons (3 of 16). Therefore, 350 herds remained in the study through completion.

Sample Collection

The sampling and mailing procedures routinely used by the NDHRS were followed. The samples were usually drawn between milkings during normal working hours for the dairy advisors. Envelopes for mailing quarter milk samples were distributed to the dairy advisors. Each envelope contained a box with 4 sterile 10-mL plastic tubes and a standard form for identification of farm and cow identity. The tubes were numbered according to the position of the quarter. Milk samples were drawn according to the Norwegian standard procedure. Teat ends were first well cleaned and dried. Then, 10 to 15 mL of milk was drawn and discarded, and the teat ends were scrubbed with a cotton or paper towel moistened with 70% ethanol; one towel was used for each teat. Good hygiene and dry hands during sampling were emphasized. The sample tube was filled $\frac{1}{2}$ to $\frac{3}{4}$ by 1 milking grasp. Samples were cooled in a refrigerator as soon as possible, at least within a few hours, after sampling and kept cooled until sent by mail as fast as possible to the mastitis laboratory. Samples drawn at the end of the week were kept cool by the sampler and were sent by mail to the laboratory on Monday. Samples arriving at the laboratory on Saturday were cooled at

the laboratory until processing on Monday, thus avoiding an uncontrolled cooling chain for >24 h.

Analyses of Milk Samples

The samples were analyzed for bacterial growth from 0.01 mL of milk spread on blood agar plate (Blood Agar Base, Oxoid Ltd, Basingstoke, UK) mixed with 5% washed bovine erythrocytes. The examination of bacterial growth and diagnostics followed the official Norwegian procedure of the National Veterinary Institute (1993) and was in agreement with the recommendations of the International Dairy Federation (1981). The plates were sectioned into 4 quadrants and incubated for 18 to 24 h at $37 \pm 1^\circ\text{C}$. The samples were judged as contaminated if there was rich growth of >2 different types of colonies. If >2 quadrants were classified as contaminated, the advisors were asked to take new samples from the same cow as soon as possible. Typical colonies for *Staphylococcus* spp. that produced a typical β -hemotoxic zone were recorded as *Staph. aureus*. The combined growth of *Staph. aureus* and *Streptococcus dysgalactiae* was classified separately (code 70). Other colonies of *Staphylococcus* spp. were classified as coagulase positive or negative using Prolex Staph Latex Kit (Pro-Lab Diagnostics, Toronto, ON, Canada). Coagulase-positive *Staphylococcus* spp. were classified as *Staph. aureus*, and *Strep. dysgalactiae* were identified by colony morphology, hemolysis, negative CAMP, and esculine reaction. *Streptococcus uberis* were identified by colony morphology, hemolysis, CAMP, esculine reaction, no growth on lactose or saccharose agar, and a positive inuline reaction. Other *Streptococcus* spp. were classified based on colony morphology, hemolytic properties, CAMP reaction, and fermentation of esculine, and they were grouped according to Lancefield's system group A, B, C, D, F, and G. Growth of 1 to 3 cfu or 4 to 15 cfu of *Staph. aureus*/0.01 mL was classified separately according to the Norwegian standard. A level of >4 cfu/0.01 mL of other major udder pathogens in pure culture was designated as positive. Bacteria isolates other than *Staph. aureus*, *Strep. dysgalactiae*, or group B or G *Streptococcae* were recorded as minor mastitis pathogens. In accordance to Nordic recommendations for mastitis analysis of quarter milk samples (Klastrup and Schmidt Madsen, 1974), minor pathogens (code >14 except 70 and 71 in Table 1) were recorded when identified in pure culture at >40 cfu/0.01 mL per quadrant of the agar plate. All quadrants with isolates of *Staph. aureus*, CNS, *Strep. dysgalactiae*, *Strep. uberis*, and coliforms were selected for examination for antibiotic resistance using 3 to 5 random cfu from the agar plate. Because of missing test results of 2 isolates from 1 cow for *Staph. aureus* and CNS, 1

isolate for coliforms, 8 isolates from 3 cows for *Strep. uberis*, and 20 isolates for *Strep. dysgalactiae*, the total material for complete resistance testing was 1,155 isolates of *Staph. aureus*, 460 isolates of CNS, 135 isolates of *Strep. dysgalactiae*, 43 isolates of *Strep. uberis*, and 41 isolates of coliforms. Susceptibility patterns of penicillin, streptomycin, and tetracycline were determined following the official Norwegian procedure (National Veterinary Institute, 1993), which is in accordance with the International Dairy Federation's procedures (1981), using the diffusion agar method on Müller Hinton agar (Difco, Sparks, MD) and Neo-Sensitabs (A.S. Rosco, Taastrup, Denmark). All isolated pathogens were frozen for future investigations on antibiotic resistance factors. The bacterial results were kept confidential until January 1, 2001, to prevent the biasing of the results by treatment or management routines that might be prompted by this information.

Statistical Analyses

Data were compiled using the SAS software (version 8.02; SAS Institute, Inc., Cary, NC). Both descriptive and multivariable statistical analyses were performed. Different multivariable models were constructed using Proc Genmod. Cow and herd were introduced as random variables when the data were analyzed at the quarter level. Herd and county were introduced as random variables when analyzing data at the cow level. The test day was transformed to the 2 variables sine (test day) and cosine (test day) by the following equations in accordance with the method used by Schukken et al. (1992) to model seasonal associations on BMSCC.

$$\text{Sine (test day)} = \sin [2 \times \pi \times (\text{test day}/365)] \quad [1]$$

$$\text{Cosine (test day)} = \cos [2 \times \pi \times (\text{test day}/365)] \quad [2]$$

The model for the microbiological result was as follows.

$$\begin{aligned} \text{Microbiological isolate (0, 1)} = & \\ & a + \beta_1 \text{ lactation number (1, 2, >2)} \quad [3] \\ & + \beta_2 \text{ sine (test day)} + \beta_3 \text{ cosine (test day)} \\ & + \beta_4 \text{ DIM} + \beta_5 \text{ age of sample} \end{aligned}$$

The parity was classified in parity 1, 2, or >2 as a class variable. Days in milk and age of sample were used as continuous variables. The variable time for samples to reach the laboratory was also tested in the model as a dichotomous variable classified as <3 or >2 d. The model was run with a binomial distribution. Significance was assessed by Type III and Wald χ^2 tests.

Table 1. Numbers and types of isolates reported from the laboratory for each quarter sample

Code	Type of isolate	Quarter site ¹				Total (%)
		RF	LF	RR	LR	
.	Dry quarters or empty sample	76	87	107	125	395 (2.8)
0	Mixed flora (overgrowth)	246	246	252	228	972 (6.9)
1	>15 cfu of β -toxic <i>Staphylococcus aureus</i>	149	143	141	169	602 (4.3)
2	4 to 15 cfu of β -toxic <i>Staph. aureus</i>	11	12	13	16	52 (0.4)
3	1 to 3 cfu of β -toxic <i>Staph. aureus</i>	128	116	125	124	493 (3.5)
6	Anhemotoxic <i>Staph. aureus</i>	0	0	1	0	1 (0.0)
7	<i>Arcanobacterium pyogenes</i>	0	1	0	0	1 (0.0)
10	<i>Streptococcus agalactiae</i>	0	0	0	0	0 (0.0)
12	<i>Streptococcus dysgalactiae</i>	36	26	45	48	155 (1.1)
13	<i>Streptococcus</i> Lancefield group G	0	0	1	1	2 (0.0)
15	<i>Streptococcus uberis</i>	9	10	17	15	51 (0.4)
16	α -hemolytic <i>streptococci</i>	7	6	3	6	22 (0.2)
18	Anhemolytic <i>streptococci</i>	0	0	1	0	1 (0.0)
19	<i>Enterococcus</i> species	1	0	0	0	1 (0.0)
20	<i>Escherichia coli</i> (anhemolytic)	5	3	7	11	26 (0.2)
23	Other coliform bacteria	5	2	3	6	16 (0.1)
30	CNS	102	104	137	119	462 (3.3)
31	<i>Corynebacterium</i> species	1	1	0	0	2 (0.0)
34	<i>Bacillus</i> species	2	2	2	1	7 (0.1)
44	Yeasts	0	2	1	0	3 (0.0)
48	No bacterial growth	2,749	2,768	2,670	2,655	10,842 (76.6)
52	<i>Enterobacter</i> species	0	0	0	1	1 (0.0)
70	<i>Staph. aureus</i> and <i>Strep. dysgalactiae</i>	1	0	3	3	7 (0.1)
71	<i>Staph. aureus</i> and antibiotics	1	0	0	1	2 (0.0)
96	Sour milk	9	8	8	9	34 (0.2)
99	Growth inhibitor	0	1	1	0	2 (0.0)
	Total	3,538	3,538	3,538	3,538	14,152 (100)
	Total <i>Staph. aureus</i> ²	290	271	283	313	1,157 (8.2)

¹RF = Right front; LF = left front; RR = right rear; LR = left rear.²Sum of codes 1, 2, 3, 6, 70, and 71.

The fit of the models was assessed by evaluation of deviance and change in deviance. The random cluster effects within cow and herd in this study were estimated using the alternative logistic regression method (Carey et al., 1993); herd was the subject, and cow was a subcluster using this equation at the quarter level in the SAS software.

Repeated subject = herd/logor = [4]

nest1 subcluster = cow identification

At cow level, the cluster effect within county and herd was as follows.

Repeated subject = county/logor = [5]

nest1 subcluster = herd ID

Separate models were made for each microbe species or class of species in the same way for individual cow level results. The cows were designated positive for a certain specific species when ≥ 1 of the quarters was positive for that species. The nonsignificant variables were removed one by one using a backward elimination procedure until all had $P < 0.10$. $P < 0.05$ was judged

as significant. For sine (test day) or cosine (test day), both were forced into the model if either or both were at $P < 0.10$.

RESULTS

Herds

The 350 herds with complete herd data for year 2000 had a mean herd size of 14.9 cows ranging from 4 to 104 with a median of 12.7 cows. Mean production was 6,079 (SD = 927) kg of milk/yr. Altogether, 97.5% of all cows were of the Norwegian Red Cattle breed, and an additional 1.7% were mixed breeds with Norwegian Red included. Feeding consisted mainly of grass silage covering 39.4% of the energy consumption, concentrate accounted for an additional 39.5%, and 16.9% of the energy requirements were covered by pasture. Pasture season usually runs from May or June until September or October depending on latitude. Altogether, 7.1% of the dairies used free-stall barns, and the rest used tie-stall barns; 60.1% of all farmers kept the heifers in a tie-stall system from service until 3 wk before calving. In mastitis control programs that are typical for Scandinavia, emphasis is placed on dry and clean bedding

areas, well-constructed milking machines, good milking practices with good preparation, good let down, and proper handling of clusters, whereas routine teat dipping and dry cow therapy are rarely recommended.

Drop Outs

A total of 4,645 cows were originally selected for sampling of which 463 cows dropped out. Of these cows, 336 belonged to farmers ending their production, 63 to farmers unwilling to cooperate, and 23 to herds that were not members of the animal recording any longer. Nineteen cows were from herds moved together with other herds, and 22 dropped out for unknown reasons. Additionally, samples were missing from 220 selected cows because they were dried off at sampling time, and 424 cows had missing samples for unknown reasons. A total of 3,538 sets of udder quarter milk samples were analyzed from 2,834 individual dairy cows: 2207 once, 555 twice, 67 three times, and 5 four times. Of the 3,538 randomly selected cows, 783 were replacement cows that were included because the selected cow was culled at the time of sampling.

Parity and DIM. There was no information regarding parity for 37 of the sampled cows. The distribution of parities was 41.0% first parity, 26.9% second parity, 16.3% third parity, 8.6% fourth parity, 4.0% fifth parity, and 3.1% ≥ 6 parity. Twenty-five percent of the samples were taken before 73 DIM, 50% were taken before 154 DIM, and 75% were taken before 234 DIM. The mean day of sampling was 162 d (SD = 119 d) postcalving. Only 2.5% had a sampling day later than 372 DIM. These figures were truncated in later analyses to 372 d because of some extreme values.

Age of Sample. Forty percent of the samples arrived at the laboratory within 1 d after sampling, 72% arrived within 2 d, and 83% arrived within 3 d. The multivariable model found no significant effect caused by sample age on the microbiological results. The samples were analyzed at one laboratory and the final diagnoses were made by only 2 veterinarians; one of them had responsibility for 91.1% of the diagnostics.

Key Microbiological Findings

Information regarding microbiological diagnoses from 13,757 quarters was obtained. Dry quarters accounted for 317 samples, and empty tubes that had been broken or experienced other accidental events during transport accounted for 78 samples for which no information was obtainable. Table 1 presents the number of isolates from the quarter samples. No microbiological growth occurred in 76.6% of the quarters. The main microbiological findings were as follows: 1,157 (8.2%)

Staph. aureus, 972 (6.9%) were contaminated samples, 462 (3.3%) coagulase-negative *Staphylococci* spp. (designated as CNS), 162 (1.2%), *Strep. dysgalactiae*, 51 (0.4%) *Strep. uberis*, and 26 (0.2%) *Escherichia coli*.

Of the *Staph. aureus* isolates, 602 (4.3% of all samples) had >15 cfu/mL, 52 (0.4%) showed moderate growth (4 to 15 cfu/mL), and 493 (3.5%) showed <4 cfu/mL after 24 h of incubation. There was no identification of *Streptococcus agalactiae*, *Pasteurella* spp., *Listeria monocytogenes*, or *Bacillus cereus*.

Resistance

Staphylococcus aureus (n = 1,155) were penicillin G resistant (PGR) in 11.4%, resistant for streptomycin in 4.8%, and resistant to tetracycline in 0.7% of the isolates. The results for CNS (n = 460) were 36.1, 19.9, and 2.6% for PGR, streptomycin, and tetracycline resistance, respectively; for *Strep. dysgalactiae* (n = 135), the corresponding figures were 0.0, 7.4, and 1.5%. For *Strep. uberis* (n = 43), PGR was found in 11.6% of the samples, 33.3% were resistant to streptomycin, and 4.8% were resistant to tetracycline, and for *E. coli* and other coliforms (n = 41) resistance was 100.0, 29.3, and 14.6%, respectively. For *Staph. aureus*, only 8 isolates (0.7%) were resistant or partially resistant for both penicillin G and streptomycin. Similarly, for *Strep. uberis*, 2 isolates (5.7%) had resistance to both antibiotics, and for CNS, 47 isolates (10.2%) had resistance to both antibiotics. Table 2 shows the number of udder quarters and cows with PGR isolates of *Staph. aureus* or CNS for each quartile of the year. A higher number of resistant isolates were identified during the period from April to June, especially for CNS. This trend was extended into July to September for *Staph. aureus*.

The overall prevalence of udder sample sets (cow level) with any isolate of *Staph. aureus* was 22.2%, for *Strep. dysgalactiae* was 3.9%, for CNS was 10.3%, and for *Strep. uberis* was 1.0%. Cows with PGR *Staph. aureus* included 2.8%, whereas PGR CNS was identified in 3.9% of cows. The number of quarters with isolates of the same microorganism at the cow level is presented in Table 3.

Seasonal Association

The observed prevalence of isolation of *Staph. aureus* over the 12-mo investigation period is illustrated in Figure 1. The highest prevalence for *Staph. aureus* was found from May to July, for CNS was found from April to July, for *Strep. dysgalactiae* was found from April to June, for *Strep. uberis* was found from May to July (Table 2; Figure 2), and for other *Streptococcus* was found from January and April (not illustrated). The

Table 2. Number of quarters and cows with findings of penicillin G resistant (PGR) *Staphylococcus aureus* or coagulase-negative staphylococci (CNS) in each quarter of the year. Prevalence¹ (%) of all samples is presented in parentheses

Annual quarter indicated by months	At quarter sample level			At cow level ²		Total number of quarter-set samples at cow level
	<i>Staph. aureus</i>	CNS	Total number ³	<i>Staph. aureus</i>	CNS	
Jan–Mar	27 (0.82)	31 (0.94)	3,305	19 (2.15)	27 (3.05)	884
Apr–Jun	39 (1.25)	94 (2.81)	3,113	31 (3.48)	76 (8.54)	890
Jul–Sep	39 (1.43)	88 (0.58)	2,725	28 (3.59)	27 (3.46)	781
Oct–Dec	27 (0.74)	38 (0.21)	3,642	20 (2.03)	9 (0.92)	983
Total	132 (1.03)	251 (1.19)	12,785	98 (2.77)	139 (3.93)	3,538

¹Prevalence is defined as the number of PGR-positive isolates divided by the total number of quarters or quarter-set samples at the cow level within the same 3 mo of the year, multiplied by 100.

²Total of 3,538 quarter sets from 2,834 randomly selected individual cows sampled ≥ 1 time. One positive quarter sample from at least 1 of 4 quarters was defined as positive at the cow level.

³Total number of quarters, except 395 dry quarters and 972 quarters with overgrowth.

association with season was independent of the classification according to number of cfu of *Staph. aureus* that were identified in a sample.

Association with DIM

Table 4 shows the prevalence of different bacterial isolates when the lactation stage was divided in 4 equal lengths. There is a decrease in the prevalence of *Staph. aureus* throughout lactation and an increase of *Strep. dysgalactiae*. Table 5 shows the prevalence of *Staph. aureus* according to sampling time split on parity and lactation stage combined. *Staphylococcus aureus* was most prevalent in the early stage of lactation in first lactation cows and lowest in second lactation cows. First and second parity cows had a decrease in the observed prevalence of *Staph. aureus* throughout lactations.

Multivariable Models with Cluster Effects Included

All models had a significant fit assessed by change in deviance; the larger the change, the better. The models

with bacteria isolates at the quarter level (Table 6) had better fit than the models at the cow level (Table 7). In general, models with *Staph. aureus* and CNS had the best fit according to change in deviance, and those with *Strep. uberis* did not have as good a fit. However, the overall best fit was found for the model with CNS at the cow level (Table 7) and for the model with PGR CNS for cows exclusively with positive isolates of CNS (Table 8).

Staphylococcus aureus. The model for *Staph. aureus* isolates in milk at the quarter level identified a significant association of test day (season) and DIM with the risk of *Staph. aureus* isolation (Table 6). The association of parity with isolation of *Staph. aureus* was close to significant for milk at the quarter level (Table 6), but was not significant at the individual cow level (Table 7). The cluster effect for *Staph. aureus* was greatest within cow, yielding an estimate of 1.58 (1.41 to 1.75) that corresponds to an odds ratio (OR) of $e^{1.58}$ or OR = 4.9 (4.1 to 5.8). The within-herd cluster effect corresponds to an OR of 1.7 (1.4 to 2.0). Figure 2 shows

Table 3. The number of 4-quarter sample sets at the cow level (percentage in parentheses) with total numbers of quarters on each cow with the same bacterial isolates

Microbe	Cows [no. (% ¹)]	Isolates per positive cow (no.)	Quarters with the same classified isolates within the same udder sample set [cow level; no. (% ²)]			
			1	2	3	4
<i>Staphylococcus aureus</i>	786 (22.2)	1.47	540 (68.7)	147 (18.2)	73 (9.3)	26 (3.3)
PGR ³ <i>Staph. aureus</i>	98 (2.8)	1.65	80 (81.6)	7 (7.1)	6 (6.1)	5 (5.1)
<i>Streptococcus dysgalactiae</i>	139 (3.9)	1.17	122 (87.8)	12 (8.6)	4 (2.9)	1 (0.7)
<i>Streptococcus uberis</i>	36 (1.0)	1.42	26 (72.2)	7 (19.4)	1 (2.8)	2 (5.6)
CNS	364 (10.3)	1.27	285 (78.3)	63 (17.3)	13 (3.6)	3 (0.8)
PGR CNS	139 (3.9)	1.19	115 (82.7)	21 (15.1)	3 (2.2)	0 (0.0)

¹Percentage of all 3,538 sample sets from the 2,834 individual cows sampled randomly with ≥ 1 quarter with a positive isolate within the same quarter set.

²Percentage of sample sets with 1, 2, 3, or 4 equal isolates within each sample set of 4 quarters.

³PGR = Penicillin G resistant.

Figure 1. Rolling 3-mo means of observed prevalence (■) at the quarter level and upper and lower confidence intervals (CI; —) for isolated *Staphylococcus aureus*. Month 1 in rolling mean is mean of months 11, 12, and 1, etc.

the association of different pathogen isolates with season estimated from the multivariable models. There was no identified within-county cluster effect.

Penicillin G-Resistant *Staphylococcus aureus*. The multivariable models confirm the significant asso-

ciation of season on PGR *Staph. aureus* both in the quarter level model (Table 6) and in the cow level model (Table 7). The same association was observed for PGR *Staph. aureus* and DIM. However, when cows exclusively with *Staph. aureus* were included in the multi-

Figure 2. Modulated seasonal trends and relative risks (RR) at the quarter level for *Staphylococcus aureus* (●) and *Streptococcus dysgalactiae* (▲) on the left axis and coagulase-negative staphylococci (CNS; □) and *Streptococcus uberis* (◇) on the right axis.

Table 4. Prevalence¹ (%) of specific bacteria isolated from quarters during each quarter of lactation

Stage in lactation	<i>Staphylococcus aureus</i>	<i>Streptococcus dysgalactiae</i>	<i>Streptococcus uberis</i>	Other <i>Streptococci</i>	Coliforms	CNS	No. of isolates	Mixed flora	Dry quarters ²	No. of quarters
<75 DIM	10.0	0.95	0.34	0.28	0.22	3.07	74.6	7.49	2.60	3,580
75 to 155 DIM	8.7	1.06	0.26	0.06	0.43	3.00	77.1	6.31	2.97	3,500
156 to 234 DIM	7.5	1.10	0.38	0.17	0.14	3.04	78.1	6.20	3.07	3,452
>234 DIM	6.5	1.50	0.48	0.20	0.40	3.98	77.0	7.13	2.57	3,464
Missing DIM	8.3	0.64	0.64	0.64	0.00	2.56	71.2	14.10	1.92	156
Total	8.2	1.14	0.36	0.18	0.30	3.26	76.6	6.87	2.79	14,152

¹Prevalence = (Number of positive quarters/total number of quarters within each stage of lactation) × 100.

²Also included empty tubes.

variable model, no association was apparent for any of the covariates other than the random effect within herd (Table 8). There was a significant association between PGR *Staph. aureus* and parity for quarter level results [OR = 1.9 (1.1 to 3.4)]. This association was not significant for the cow level model (Table 7). There was no significant within-cow cluster effect for PGR *Staph. aureus*; however, a very strong within-herd cluster effect was observed [OR = 8.8 (4.5 to 16.9)].

CNS. The model with CNS as a dependent variable confirmed a highly significant seasonal association both for the quarter level model (Table 6) and for the cow level model (Table 7). There was no association between CNS and DIM. There was a borderline significant association between isolation of CNS (Table 7) and parity >2 compared with parity 1 [OR = 1.3 (1.0 to 1.8)]. There was a strong within-cow cluster effect [OR = 4.7 (3.5 to 6.3)] and a corresponding significant within-herd cluster effect [OR = 1.4 (1.2 to 1.6)] for the udder quarter model. The model with only CNS included revealed a significant association between PGR resistance and both season and DIM (Table 8). However, parity or the within-herd cluster effect was not significantly associated with the likelihood of PGR in CNS.

***Streptococcus dysgalactiae*.** The model with *Strep. dysgalactiae* illustrated a nonsignificant association both for season ($P = 0.06$ and 0.16) and DIM ($P = 0.07$) for the quarter level results (Table 6). This was shown to be significant in the model at cow level (Table 7). There was a strong association of isolates with *Strep.*

dysgalactiae and parity: OR = 3.0 (1.9 to 4.8) comparing parity >2 with first parity and OR = 1.9 (1.1 to 3.0) comparing second parity with first parity cows. There was a highly significant within-cow clustering [OR = 10.2 (6.4 to 16.3)]. There was also a within-herd cluster effect in the cow model [OR = 1.6 (1.0 to 2.5)].

***Streptococcus uberis*.** The model with *Strep. uberis* illustrated an association with season both at the quarter and cow levels (Tables 6 and 7). There were no parity, DIM, or within-cow cluster associations identified. However, there was a significant within-herd cluster association [OR = 11.2 (6.2 to 20.3)].

DISCUSSION

This survey was constructed to acquire important information regarding microbiological prevalence in quarter milk samples in Norway during 2000. As in all field studies, we expected some natural, unavoidable dropouts, such as farmers leaving the dairy business. This reflected the overall percentage of producers dropping out of production and selling their quota at that time. Bias caused by dropout may be associated with producers with low milk quality or high infection levels being lost to follow up. Farmers refusing to join the project constituted only 1.4% of the total, which indicates that the survey response rate was very high and should not have biased the results. Samples not taken for other reasons accounted for 9.1% of the samples. This was most common during the summer when the

Table 5. Number of quarters and prevalence¹ (%; in parentheses) of identified *Staphylococcus aureus* according to DIM and parity number

Parity	Missing DIM	<74 DIM	73 to 155 DIM	154 to 234 DIM	>234 DIM	Quarters [no. (%)]
1	16 (0.0)	1,224 (11.4)	1,432 (8.5)	1,476 (8.3)	1,596 (6.2)	5,744 (8.4)
2	0 (—)	1,120 (8.6)	904 (8.0)	880 (6.3)	860 (5.9)	3,764 (7.3)
3	0 (—)	624 (10.5)	624 (8.3)	536 (5.6)	504 (7.7)	2,288 (8.0)
>3	0 (—)	608 (9.9)	536 (10.6)	560 (9.1)	504 (7.3)	2,208 (9.3)
Missing parity	140 (9.3)	4 (0.0)	4 (0.0)	0 (—)	0 (—)	148 (8.8)
Total number	156 (8.3)	3,580 (10.0)	3,500 (8.7)	3,452 (7.5)	3,464 (6.5)	14,152, (8.2)

¹Prevalence = (Number of positive quarters with isolates of *Staph. aureus*/total number of quarters within each stage of lactation) × 100.

Table 6. Estimated results from the multivariable models [Proc Genmod (SAS Institute, Inc., Cary, NC); with cluster effects] of quarters with *Staphylococcus aureus*, penicillin G-resistant (PGR) *Staph. aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and CNS as the dependent variable. Δ = Change in deviance for full model vs. no covariates in the model; CI = confidence interval

Fixed and random variables	<i>Staph. aureus</i>		PGR <i>Staph. aureus</i>		<i>Strep. dysgalactiae</i>		<i>Strep. uberis</i>		CNS	
	β or Δ	CI of β	β or Δ	CI of β	β or Δ	CI of β	β or Δ	CI of β	β or Δ	CI of β
Δ Model	94.0		35.7		39.6		44.4		175.4	
Intercept	-2.27***	-2.46 to -2.09	-4.59***	-5.18 to -4.00	-5.37***	-5.88 to -4.86	-6.16***	-6.7 to -5.6	-3.71***	-3.9 to -3.5
Parity 1	0.15 ¹	-0.04 to 0.34	0.11 ^{NS}	-0.45 to 0.68	0				0	
Parity 2 ²	0		0		0.78**	0.28 to 1.28			0.20 ³	-0.02 to 0.42
Parity >2	0.15 ⁴	-0.04 to 0.34	0.65*	0.08 to 1.23	1.01***	0.54 to 1.48				
Sine (test day)	0.010	-0.10 to 0.11	-0.036	-0.42 to 0.35	0.23 ⁵	-0.007 to 0.47	-0.112	-0.74 to 0.51	0.69***	0.54 to 0.85
Cosine (test day)	-0.32***	-0.46 to -0.18	-0.67**	-1.12 to -0.22	-0.20 ⁶	-0.48 to 0.08	-1.58**	-2.5 to -0.66	-0.60***	-0.82 to -0.37
DIM	-0.0018***	-0.0026 to -0.0010	-0.0030*	-0.0055 to -0.0005	0.0017 ³	-0.0002 to 0.0035				
Within cow ⁷	1.58***	1.41 to 1.75			2.32***	1.86 to 2.79			1.54***	1.24 to 1.84
Between cows ⁷	0.51***	0.33 to 0.70	2.17***	1.51 to 2.83	0.40*	-0.0078 to 0.82	2.43***	1.84 to 3.01	0.33***	0.16 to 0.50

¹ $P = 0.13$.

²For the model of CNS, parity 2 = all parities >1.

³ $P = 0.07$.

⁴ $P = 0.12$.

⁵ $P = 0.06$.

⁶ $P = 0.16$.

⁷Random variable.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 7. Estimated results from the multivariable models [Proc Genmod (SAS Institute, Inc., Cary, NC); with cluster effects] for cows with *Staphylococcus aureus*, penicillin G-resistant (PGR) *Staph. aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and CNS as dependent variables. Δ = Change in deviance for full model vs. no covariates in the model; CI = confidence interval

Fixed and random variables	<i>Staph. aureus</i>		PGR <i>Staph. aureus</i>		<i>Strep. dysgalactiae</i>		<i>Strep. uberis</i>		CNS	
	β or Δ	CI of β	β or Δ	CI of β	β or Δ	CI of β	β or Δ	CI of β	β or Δ	CI of β
Δ Model	45.1		11.0		32.2		13.3		93.4	
Intercept	-0.85***	-1.0 to -0.67	-3.29***	-3.7 to -2.9	-4.19***	-4.7 to -3.7	-4.8***	-5.3 to -4.3	-2.4***	-2.7 to -2.2
Parity 1					0				0	
Parity 2					0.62*	0.12 to 1.11			0.20 ^{NS}	-0.09 to 0.50
Parity >2					1.10***	0.63 to 1.57			0.29*	0.001 to 0.58
Sine (test day)	0.07	-0.05 to 0.19	0.15	-0.19 to 0.49	0.05	-0.23 to 0.32	0.04	-0.44 to 0.51	0.59***	0.41 to 0.76
Cosine (test day)	-0.26***	-0.40 to -0.12	-0.49**	-0.85 to -0.12	-0.33*	-0.64 to -0.02	-1.12**	-1.94 to -0.31	-0.62***	-0.86 to -0.38
DIM	-0.0026***	-0.004 to -0.002	-0.0025*	-0.005 to 0.00	0.0022*	0.0002 to 0.0041				
Within herd ¹	0.46***	0.23 to 0.70	1.35***	0.63 to 2.06	0.47*	0.013 to 0.92	1.56***	0.79 to 2.34	0.39***	0.17 to 0.61

¹Random variable.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 8. Estimated results from the multivariable models [Proc Genmod (SAS Institute, Inc., Cary, NC); with cluster effects] when only those cows with *Staphylococcus aureus* or CNS were included in the model and with penicillin G-resistant (PGR) *Staph. aureus* or PGR CNS, respectively, as the dependent variable¹

Fixed and random variables	PGR CNS		PGR <i>Staph. aureus</i>	
	β or Δ	CI of β	β	CI of β
Δ Model	25.2			
Intercept	-1.47***	-2.13 to -0.80	-2.06***	-2.4 to -1.77
Parity 1	0			
Parity 2	0.57 ²	-0.03 to 1.16		
Parity >2	0.38 ^{NS}	-0.17 to 0.92		
Sine (test day)	0.16	-0.22 to 0.56		
Cosine (test day)	-0.58**	-0.94 to -0.22		
DIM	0.0036**	0.0009 to 0.0062		
Within herd ³	0.55 ⁴	-0.09 to 1.19	1.37***	0.55 to 2.18

¹ Δ = Change in deviance for full model vs. no covariates in the model; CI = confidence interval.

² P = 0.06.

³Random variable.

⁴ P = 0.09.

* P < 0.05; ** P < 0.01; *** P < 0.001.

herd was out to pasture and people were on vacation or having other higher priority tasks. Overall, there was balanced representation in the total sample with regard to parity and DIM. The distribution of cows being sampled 2, 3, and 4 times was in accordance with the random sampling strategy in the study. The distribution of parities was very similar to the distribution within the whole population: 40.3% were sampled in first parity, 26.3% were sampled in second parity, 16.6% were sampled in third parity, and 9.2% were sampled in the fourth parity (TINE Norwegian Dairies, 2001). This also demonstrated that the substitution system for selected cows that had been culled worked well and resulted in the proper number of first parity cows in early lactation. This was also demonstrated by the even distribution of cows throughout DIM. The correct geographical and parity distributions, with only 1.4% unwanted dropouts, gave assurance that the material consisted of a true random sample from the Norwegian dairy cattle population within the recorded year.

All microbiological testing was done in one laboratory to ensure that the quality of diagnostics and methods was consistent. Most of the samples (83.2%) arrived at the laboratory by the third day after sampling. All samples were kept cool by either the sampler or the laboratory, so that interruption of the cooling-chain should not have exceeded 24 h. The statistical models were fit with age of sample as an independent variable, and age was found to have no significant contribution. To be sure that the association with season and pathogen isolates was not due to older samples having more growth during the summer, separate models were constructed for one data set with samples arriving at the laboratory in <3 d and another data set with samples delivered to the laboratory at >2 d after collection. The

results of these models were not significantly different. Thus, the 16.8% of samples that were >2 d in transport from the cow to the laboratory should not have biased our results. Some areas of the country might have longer transport distances and need all working days to draw all of the samples; their samples were included in the material to avoid geographical selection bias.

Nordic recommendations on mastitis diagnostics according to Klasttrup and Schmidt Madsen (1974) with a limit of 40 cfu/0.01 mL was used as a cut-off for diagnosis of minor pathogens. This could cause a slight underestimation of these bacteria (code 15 to 44 and 52 in Table 1) in relation to the results presented by Pitkälä et al. (2004).

We tested antibiotic resistance by agar diffusion, which is the standard method for screening antibiotic resistance used in Norway. The study would have benefited from the use of the minimum inhibitory concentration method used by Pitkälä et al. (2004) in Finland, but the resources required were not available. The resistant strains were stored, and future studies should provide further insight into the type of bacterial resistance genes present. The diameter limits for resistance in the disc method used in this study were defined by values for minimum inhibitory concentration supplied by the manufacturer. Therefore, our results should be comparable with the results in the paper by Pitkälä et al. (2004) in Finland, although probably with less precision.

The predominant organisms isolated were *Staph. aureus*, CNS, and *Strep. dysgalactiae*. This result agrees with findings from earlier years from routine samples analyzed at the Norwegian Mastitis Laboratories. The only significant difference from the routine samples was the identification of a lower percentage of

PGR *Staph. aureus* and CNS in the current study, which might be explained by more frequent, routine follow-up sampling in herds in which resistance has been revealed previously.

Very few surveillance studies have been done on a country-wide basis with the exception of one recent study from Finland (Pitkälä et al., 2004). The biggest difference between our study and theirs was a much lower fraction of CNS reported in Norway than in Finland: 3.3 vs. 16.6%, respectively. The present study also found a high prevalence of *Staph. aureus* (8.2 vs. 3.4%). However, we recorded all *Staph. aureus*, even if present with only 1 cfu/0.01 mL of milk. If we restrict our findings to >15 cfu of *Staph. aureus*, which would be more comparable with that reported from Finland, our calculated prevalence would be 4.3%. The prevalence of *Strep. uberis* was low both in Norway and Finland studies (0.4 vs. 0.65%). This was in contrast to reports from the United States and the United Kingdom (Zadoks et al., 2004; Green et al., 2005). We did not identify any *Corynebacterium bovis*. This was expected, as *C. bovis* is very rarely isolated from cows in Norway. Pitkälä et al. (2004) reported a prevalence of *C. bovis* of 11.52% in Finland. Barkema et al. (1997) found a very high intraclass correlation for *C. bovis*, at the same magnitude as that of *Streptococcus agalactiae*, demonstrating a high degree of contagiousness. If *C. bovis* are contagious bacteria, as indicated by Barkema et al. (1997), this could support the hypotheses that *C. bovis* are still not very common nor spread in the Norwegian population yet. Another hypothesis is that the Norwegian cattle population's environment is not conducive to *C. bovis* infection. Our study reported that 76.6% of the quarters were classified as having no bacterial growth, whereas 62.4% were so classified in Finland. The proportion of PGR *Staph. aureus* in the current study was 11.4%, but was 52.1% in Finland. We also found less resistance to tetracycline in our *Staph. aureus* isolates (0.7%), whereas Finland's finding was 5.1%. However, resistance to streptomycin was very similar (4.8% in Norway and 4.1% in Finland).

There were also country-wide surveillance data collected in The Netherlands in 1973, 1975, 1980, and 1985 (Vecht et al., 1987). Those researchers reported a level of 6% for *Staph. aureus* isolations from 1973 and 1975, 3.7% from 1980, and 4% from 1985. This was approximately the same result that we obtained, if a positive result is defined as a bacterial count of >15 cfu of *Staph. aureus*. Vecht et al. (1987) also report 1 to 1.5% isolation rates for *Strep. dysgalactiae* and *Strep. uberis*, separately. Their result for *Strep. dysgalactiae* was similar to ours, but the rate of *Strep. uberis* in their study was much higher than that in the current report.

Busato et al. (2000) from Switzerland reported a *Staph. aureus* prevalence of 16.0% upon examination of quarters during 7 to 100 DIM and 7.4% during 101 to 305 DIM. Thus, *Staph. aureus* seems to be at a higher level in Switzerland during the first part of lactation than it is in Norway, but at the same level later in lactation.

Our survey suggests that the goal for the future will be to decrease the prevalence of *Staph. aureus* by testing different options such as teat dipping and dry cow therapy. Additionally, we need to look more closely at the reason for the high prevalence of *Staph. aureus* at calving, especially in first-parity cows or heifers. Preventive measures designed to reduce known risk factors, such as cross-suckling between calves, transmission by flies under pasture conditions (Owens et al., 1998; DeVlieghe et al., 2004), and udder edema around calving (van Dorp et al., 1999; Waage et al., 2001), could be of importance. Further research needs to be done to quantify more exactly the importance of these factors under current conditions in Norway.

Number of Positive Quarters per Cow and Clustering. Table 3 shows that *Staph. aureus* was found in most quarters within the same cow if any *Staph. aureus* was isolated from a cow. This indicates that, for *Staph. aureus*, the transmission of bacteria is highest between quarters and within cows. The cluster effect within herds was largest for PGR *Staph. aureus*. This was linked to certain herds, indicating that resistance could be due to certain strains of bacteria existing within certain herds. This indicates that it could be important to avoid transferring such strains between herds. Such transmission in Norway has been previously described (Waage et al., 2002). County was introduced as a random effect to determine whether there was any geographical clustering of pathogens or PGR within the county as a consequence of animal trading between herds. Such clustering could also be generated because of different incidences of mastitis treatments between counties (Norwegian Cattle Health Services, 2004). However, no such clustering was identified. *Streptococcus uberis* also showed a large clustering effect within herd. This is in contrast to the findings of Barkema et al. (1997), who found very low within-herd correlation for *Strep. uberis*. However, our data set on *Strep. uberis* is very small, and care should be taken when interpreting our finding on this species. This indicates that either a specific strain was involved in the herds in this study or a specific environment within the herds contributed to the mechanism of infection. This hypothesis of specific strains is in accordance with the results of Zadoks et al. (2003). The model with only CNS- or *Staph. aureus*-positive cows (Table 8) showed a much larger cluster effect for PGR with *Staph. aureus* than

with CNS. This indicates that there may be different mechanisms of transmission of resistance in these 2 bacteria. Resistance genes can be either incorporated into the bacterial genome or into plasmids (Yazdankhah et al., 2000). *Staphylococcus aureus* occurs more commonly in certain herds than does CNS, which hardly demonstrated any significant herd-level clustering effect. This is not in accordance with Barkema et al. (1997), who found very similar and moderate within-herd correlations of 0.05 and 0.07 for *Staph. aureus* and CNS, respectively. This could indicate that different strains of these pathogens exist in Norway than in The Netherlands. The *Staphylococcus aureus* strain differences among the Nordic countries have been described by Aarestrup et al. (1997).

Season. There was a very strong seasonal association of isolates for all bacteria as well as for resistance. The highest prevalence of *Strep. dysgalactiae* and CNS was found in the late indoor season between April and May in Norway, and the prevalence of *Staph. aureus* and *Strep. uberis* peaked during the period from June to July. The models with *Strep. uberis* or *Strep. dysgalactiae* as dependent variables did not have as good a fit as the models for *Staph. aureus* or CNS. This could have been due to the much lower positive numbers for *Strep. uberis* or *Strep. dysgalactiae*. The higher proportion of infected cows or quarters detected in the late indoor season could be linked either to reduced resistance caused by lower feed quality or to some climatic or hygienic conditions causing higher incidence or longer duration of infections. Feed quality may be linked to lower levels of vitamin E in stored feed. Vitamin E is known to have an impact on resistance to bacterial infections (Allison and Laven, 2000). The climatic or hygienic conditions should be similar during spring and winter, as cows are kept indoors during that time. However, during spring, there may be increased sunshine, which may increase the indoor temperature. Future research is needed to determine the exact reason for these differences. The peak in prevalence of *Staph. aureus* shown in Figure 2 might have been expanded to the right because of more persistent infections or higher new infection rates. Figure 2 also indicates that the peak of *Staph. aureus* prevalence is broader than the peak of CNS, nicely illustrated by the flat area from August to December for CNS. The average duration of infection is not significantly longer for *Staph. aureus* than for CNS (Lam et al., 1997), but some clone types of *Staph. aureus* cause more persistent infections (Haveri et al., 2005). Thus, the duration of infection could be linked more to clone type than to species, indicating that the late indoor season could also have an impact on *Staph. aureus* infections. *Streptococcus uberis* has a very distinct summertime peak. This result may be due

to summer pasturing problems. Interestingly, Norway has a much lower *Strep. uberis* prevalence than other countries with much longer outdoor seasons. Norway has also fewer free-stall barns than other countries. Additionally, bedding material such as straw is infrequently used in many counties in Norway, and often a thin layer of sawdust on concrete or rubber mats is used as the only bedding material. Because streptococcal counts have been shown to be higher in chopped straw than in sawdust (Hogan et al., 1989), this could be one of the reasons why *Strep. uberis* is less frequently detected in Norway. The summer peak suggests that infections with *Strep. uberis* are more linked to climatic conditions, pasturing conditions, and the environment than are other bacteria in Norway. This corresponds very well to reports from New Zealand, where *Strep. uberis* contamination of paddocks and muddy races increased when wet conditions prevailed and when the cows' grazing density was higher (Lopez-Benavides et al., 2005). The Norwegian grazing season can be very wet, at least near the coastline, with muddy paddocks and races not uncommon during cold, wet weather.

The most surprising and new finding in this study was the huge seasonal association of the prevalence of PGR, both in *Staph. aureus* and CNS. As to PGR *Staph. aureus*, this is mainly due to higher prevalence of *Staph. aureus* during the summer as a whole, but for PGR CNS, there was also an additional increase caused by higher proportional rate of PGR CNS during the late indoor season. This is demonstrated by significant association of season and PGR CNS when the model is constructed only for quarters and cows positive for *Staph. aureus* and CNS, respectively. This finding potentially reflects different underlying mechanisms for development of occurrence of PGR in *Staph. aureus* compared with CNS. The reason for this finding is unknown; however, it was so characteristic that it will be important to investigate it in future studies. To our knowledge, there is, at present, no information in the bovine mastitis literature on the seasonal occurrence of PGR. However, such seasonality is for pneumococcal isolates causing acute otitis media in children (Hoberman et al., 2005). One hypothesis is that the transmission of resistance genes is dependent on temperature, the environment, or both, such that Norwegian summer conditions favor such transmission. This study at least illustrates large dynamics in the prevalence of PGR. According to mathematical models of infection dynamics (Kleinbaum et al., 1982), this means that incidence, duration, or both are also dynamic. The reasons for this dynamic behavior should be elucidated by future epidemiological and therapy studies that include PGR.

Association with DIM. The prevalence of both *Staph. aureus* and PGR *Staph. aureus* decreased

throughout lactation. This could have been a result of farmers culling these cows. However, in this study, the microbiological findings were not shared with the farmers. The only way these cows could be identified was through higher SCC, according to the design of the survey and the blinding of the farmers. The prevalence of *Strep. dysgalactiae* increased during DIM, indicating an accumulation during lactation or a higher risk for new infection around the end of lactation.

CONCLUSIONS

This random survey from Norway in 2000 found that *Staph. aureus*, CNS, and *Strep. dysgalactiae* are the most prevalent bacteria isolated from quarter milk samples. There was a pronounced seasonal trend, and the highest prevalence was noted during late spring for *Strep. dysgalactiae* and CNS or during summer for *Staph. aureus* and *Strep. uberis*. *Streptococcus dysgalactiae* had a tendency to accumulate during lactation. The highest risk for the presence of *Staph. aureus* occurred just after calving. *Streptococcus dysgalactiae* showed the greatest within-cow clustering effect, but *Staph. aureus* and CNS displayed a similar, substantial within-cow clustering effect. There was a large within-herd clustering effect for PGR *Staph. aureus* and *Strep. uberis*, but not for CNS. Some of the findings with regard to seasonal and lactation associations were highly unexpected, particularly the seasonal association with regard to PGR. In Norway and other locations with similar conditions, this survey will result in much more attention paid to mastitis control for *Staph. aureus* at calving and for *Strep. dysgalactiae* throughout lactation. Much more focus will be placed on preventive measures during the late indoor season for *Streptococcus* spp. and CNS and at the start of the pasture season for *Staph. aureus* and *Strep. uberis*. Future research should test the feed quality hypothesis, especially with regard to vitamin E and immune system effects during the late indoor season, as well as the reason for the association between pasturing and increased numbers of isolates of *Strep. uberis*. Thus, the present study demonstrates the importance of a random survey as a baseline for national strategic planning of mastitis control, as many of these findings could not have been discovered from routine sampling. Such sampling would likely have included selection biases. Such surveys should be repeated regularly, as in Finland where Pitkälä et al. (2004) illustrated a large change in bacteria occurrence over 10 yr. It is most probable that different prevalences of *Staph. aureus*, *Strep. uberis*, and CNS over time and between countries requires different mastitis control programs that can be adapted to the most frequent bacteria species.

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