MONITORING SUBCLINICAL MASTITIS IN DUTCH DAIRY HERDS

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World-wide the cow somatic cell count (SCC) in milk is used as an indicator of udder health. Cow SCC records are available to every farmer that participates in the milk-recording program of the Dairy Herd Improvement Association (DHIA). The bacteriological causes of increased cow SCC can only be revealed by bacteriological culturing of the milk. Culturing results can be used to take effective measures to treat and prevent (sub)clinical mastitis.

Since the end of 1996 Dutch dairy farmers have the possibility to participate in a bacteriological monitoring program (BMP) for high SCC cows, offered by the Animal Health Service. The results of this BMP give an excellent opportunity to get an overview of the bacteriological causes of subclinical mastitis in the Netherlands from 1996 till 1999.

The objective of this study was to examine the main causes of subclinical mastitis in the Netherlands and the relationships between culturing results and dairy cow characteristics.

Data Collection

During the years 1996 –1999, 3,542 dairy farmers participated in the BMP for high SCC cows. They took quarter milk samples from all or a part of the cows that had a SCC above 250,000 cells/ml for at least two of the last three milk recordings. The quarter milk samples were sent to the Animal Health Service for bacteriological culturing according to IDF procedures (1981). Isolates were classified as *Staphylococcus aureus* (SAU), *Streptococcus agalactiae* (SAG), *Streptococcus uberis* (SUB), *Streptococcus dysgalactiae* (SDY), *Arcanobacterium pyogenes* (APY), *Escherichia coli*, (ECO), *Staphylococcus non aureus* (CNS), combination of streptococci (MSTR) and negative (NEG). Isolates like *Proteus*, *Pseudomonas* spp., yeasts, *Listeria* etc. were classified as OTHER. Isolation of three or more types of pathogens in one quarter was defined as contaminated (CONT). When two pathogens were isolated in one quarter sample, the culturing result was coded according to the pathogen listed first in the following order: SAU, SAG, SUB, SDY, APY, ECO, CNS, MSTR, OTHER, NEG. This list is based on a ranking according to their assumed impact on SCC and udder health. The same ranking order was used to assign cows with more than one infected quarter to one pathogen.

Besides BMP data, monthly milk-recording data consisting of daily milk production, cow SCC, days in milk (DIM) and parity were also available.

Statistical Analysis

For cows with DHIA records, DIM at the time of culturing, parity, mean geometric SCC and mean daily milk production of all milk recordings were calculated for the current lactation. The

SCC's were transformed with the natural logarithm to obtain better statistical properties. The number of cows infected with APY and MSTR was not sufficient for statistical analysis and were therefore excluded from analysis. Also CONT cases were excluded from analysis.

Model [1] was used to estimate relationships between isolated pathogens and mean SCC in lactation, after corrections for milk production and parity. Cows that were sampled for culturing in more than one lactation, were left out of model [1], because of the interdependence of SCC between lactations. The model was fitted using the REML method (Genstat 5 Release 4.1, 1998).

Model [1]:

$$Y_{jklmnop} = \mu + HERD_j + COW_k + MILK_l + PAR_m + PATH_n + YR_o + PATH_n*(MILK_l + PAR_m + YR_o) + e_{jklmnop}$$

where: $Y_{jklmnop}$ = natural logarithm of the mean geometric SCC in the lactation (lnSCC); μ = overall mean; $HERD_j$ = random effect of herd j (j = 1...3,402); COW_k = random effect of cow k (k = 1...38,444); $MILK_l$ = fixed effect of mean daily milk yield l (l = <21, 21-26, 26-30, >30 kg/day); PAR_m = fixed effect of parity m (m = 1, 2, 3, 4, 5, ≥6); $PATH_n$ = fixed effect of isolated pathogen (n = SAU, SAG, SUB, SDY, ECO, CNS, OTHER, NEG); YR_o = fixed effect of year o (o = 1996, 1997, 1998, 1999); $e_{jklmnop}$ = random error term.

Model [2] was used to estimate relationships between prevalence of pathogens, year, parity and stage of lactation. The model was fitted using regression analysis with Poisson distribution.

Model [2]:

$$Y_{ijklmn} = \mu + 2\&3\text{-way interaction } (YR_i * SEASON_j * PAR_k * DIM_l) + PATH_m + PATH_m* (YR_i + SEASON_j + PAR_k + DIM_l) + e_{ijklmn}$$

where: Y_{ijklmn} = logarithm of number of cows; μ = overall mean; YR_i = year i (i = 1996, 1997, 1998, 1999); $SEASON_j$ = season j (j = winter, spring, summer, autumn); PAR_k = parity k (k = 1, 2, 3, 4, 5, ≥6); DIM_l = days in milk at the time of culturing (l = <100, 100-200, >300); $PATH_m$ = isolated pathogen m (m = SAU, SAG, SUB, SDY, ECO, CNS, OTHER, NEG); e_{ijklmn} = random error term.

Results

During the years 1996-1999 quarter milk samples were collected from 46,015 cows, 45,285 of which had DHIA records. Some cows were sampled more than once for bacteriological culturing, resulting in a total of 57,892 cow records. In some cases not all quarters of a cow were sampled. The total number of cultured quarters was 204,767. In Table 1 the number of quarters per pathogen, the number of animals that were assigned to a certain pathogen (including percentage of total) and the mean number of infected quarters of these cows are presented. The most prevalent pathogen was SAU, followed by SUB and CNS.

On average, rear quarters were more often infected (57%) than front quarters (43%). SUB, SDY, APY and ECO were more frequently isolated from rear quarters than from front quarters (68% vs 32% for APY and 60% vs 40% for SDY, SUB and ECO).

The percentage of quarters with SCC above 250,000 cells/ml in bacteriologically negative cows was 27.8% of all right front quarters and 25.6%, 37.0% and 36.3% of respectively left front, right rear and left rear quarters. This indicates that quarter SCC can be above 250,000 cells/ml while no pathogens can be isolated.

Table 1. The number of quarters per pathogen, the number of animals that were assigned to a pathogen and the mean number of infected quarters of these cows.

Pathogen	No. of quarters	% of quarters	No. of animals	% of animals	No. of infected quarters
SAU	21,823	10.7	15,846	27.4	1.6
SAG	1,694	0.8	865	1.5	1.9
SUB	7,239	3.5	4,986	8.6	1.4
SDY	4,007	2.0	2,574	4.5	1.3
APY	253	0.1	205	0.4	1.2
ECO	1,478	0.7	1,206	2.1	1.1
CNS	6,901	3.4	3,607	6.2	1.3
MSTR	248	0.1	126	0.2	1.1
OTHER	678	0.3	370	0.6	1.3
NEG	157,920	77.1	27,660	47.8	
CONT	2,526	1.2	447	0.8	
TOTAL	204,767	100	57,892	100	

Table 2. Estimated mean SCC for every pathogen, across milk production and parity groups (x1000 cells/ml).

Pathogen	Mean	Milk production (kg/day)				Parity					
		<21	21-26	26-30	> 30	1	2	3	4	5	≥6
SAU	372	399	369	361	354	284^{a}	317^{ab}	340^{b}	399^{c}	441^{c}	488^{c}
SAG	329	369^{a}	344^{a}	351^{a}	265^{b}	233^{a}	290^{b}	324^{bc}	344^{c}	399^{d}	424^{d}
SUB	340	372^{a}	314^{b}	344	330	237^{ab}	279^{b}	321^{bc}	365^{c}	428^{cd}	464^{d}
SDY	294	324^{a}	305^{a}	284	265^{b}	204^{a}	221^{a}	287^{b}	337^{bc}	340^{c}	437^{d}
ECO	199	257^{a}	178^{b}	194^{b}	178^{b}	123^{a}	151^{b}	171^{b}	202^{c}	245^{d}	395^{e}
CNS	270	260	257	270	290	237^{a}	245^{a}	240^{a}	287^{b}	290^{b}	330^{b}
OTHER	277	255^{a}	287	255^{a}	321^{b}	219^{a}	211^{a}	265^{b}	302^{b}	262^{b}	469^{c}
NEG	212	233^{a}	217	196^{b}	204	150^{a}	171^{a}	204^{b}	233^{bc}	255^{c}	296^{cd}

^{a,b,c,d,e} Estimated means with different superscripts within each row differ ($P<0.05$).

Since cows, which were sampled for culturing in more than one lactation were left out of the analysis, model 1 included a data set of 38,444 cows. Of all cows 28% were sampled within the first 100 DIM, 32% between 100 and 200 DIM, 24% between 200 and 300 DIM and 16% after 300 DIM. Mean daily milk production was 26.6 kilograms per day. Of all cows 21% were heifers, 20% of second parity, 19% of third parity, 15% of fourth parity, 11% of fifth parity and 14% of parity ≥6. Differences between herds explained 17% of total variance in lnSCC and 83% of total variance was explained by differences between cows. The lnSCC differed by parity, mean daily milk production, DIM at the time of culturing, isolated pathogen and year of culturing ($P<0.05$). The estimated mean SCC (x1000 cells/ml) for every pathogen, milk production and parity group is presented in Table 2. For most pathogens, the SCC was

significantly lower as the mean daily milk production was higher. Parity had a significant increasing effect on SCC for every pathogen category. In general SCC was lowest for cows infected with ECO and bacteriologically negative cows and highest for cows infected with SAU.

In model 2 all 45,285 cow records were used to examine whether the occurrence of pathogens varied across parities, DIM and years. The analysis of deviance revealed that interactions between pathogen and year, pathogen and season, pathogen and parity and pathogen and DIM were all significant ($P < 0.001$).

The occurrence of SAU showed a decrease from 31% of all isolates in 1997 to 28% in 1998 and 25% in 1999. In contrast the prevalence of negative culturing results increased over the years (45%, 47% and 50% of culturing results in 1997, 1998 and 1999 respectively). The other pathogens did not show a specific time trend in occurrence.

The prevalence of SUB increased significantly with parity. In heifers 6% of all isolates was SUB and this percentage increased to 13% of all isolates in cows of parity ≥6. The prevalence of CNS was significantly higher in heifers (11%) compared to other parities (mean prevalence 6%). This makes that SAU (29%) and CNS (11%) are the most common pathogens in heifers.

ECO and SUB occurred more often in early lactation (2.6% and 9.2%) and less often at the end of the lactation (1.4% and 8.0% for ECO and SUB respectively).

Discussion

This study gave an overview of 4 years monitoring of subclinical mastitis in the Netherlands. Although a large number of cows have been cultured, it is not a random sub sample of all cows in The Netherlands, but only those cows of interest to the farmer. The results of the present study indicate that SAU is by far the most common cause of subclinical mastitis in The Netherlands. This is also found in other European countries (Booth, 1995). Most of the samples submitted for culturing appeared to be negative. A reason for this may be that the number of colony forming units in milk is below the detection limit of the assay. This may be the case in cows infected with SAU, due to the cyclic nature of shedding pattern of this pathogen (Sears *et al.*, 1990). Another reason for the negative results may be spontaneous bacteriological recovery. It is important to monitor SCC after a negative culturing result is obtained. If the SCC remains increased, a consecutive sampling may improve the probability of an accurate diagnosis.

The present study showed that the number of infected rear quarters was higher for SUB, SDY, ECO and APY compared to the front quarters. The higher occurrence of SUB and SDY in rear quarters was also reported by Barkema *et al.* (1998), but in their study SAU and CNS were also isolated more frequently from rear quarters. They also found that right quarters were more often infected than left quarters. This was not the case in the present study (50-50%).

Results showed increasing SCC with parity, which is in accordance to a study described by Sheldrake *et al.* (1983). Besides, a relatively large part of the sampled cows were of parity ≥5 (25%). This indicates that older cows probably have more problems with udder health.

The fact that SUB was isolated more frequently in older cows compared to heifers was also reported by Jayarao *et al.* (1999). Fox *et al.* (1995) and Nickerson *et al.* (1995) also found a high isolation frequency of CNS in heifers followed by SAU. In this study SAU was more frequently isolated in heifers followed by CNS.

Conclusion

Data from the bacteriological monitoring program for high SCC cows, showed that most cows had a negative bacteriological result (47.8%). Cows with a positive bacteriological result were most often infected with SAU (27.4%). Also SUB and CNS were found in many cases (8.6 and 6.2% respectively).

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