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Dutch national mastitis survey. The effect of herd and animal factors on somatic cell count

U. Vecht[1], H. J. Wisselink[1] and P. R. Defize[2]

[1] Central Veterinary Institute, Lelystad, Netherlands
[2] Institute of Applied Computer Science (ITI-TNO), Delft, Netherlands

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Summary

The purpose of the national mastitis survey was to collect information on the prevalence and causes of bovine subclinical mastitis in the Netherlands. Milk samples were collected once from 10 336 cows in a stratified random selection of herds (n = 227) in the Netherlands during the years 1985-1986. Results showed that 84.2 % of the cows were free from mastitis. Ten per cent of all udder quarters were infected, and 3.7 % of these were infected with *Staphylococcus aureus*, the main udder pathogen. Statistical analysis based on a 'split-plot' model was used to analyse the effect of herd factors and animal factors on somatic cell counts (SCC). Several factors significantly influenced SCC: breed, season, geographical region, type of housing, and the use of teat disinfection. The effect of herd and animal factors on SCC of milk samples of individual cows was calculated as deviation from the geometric mean cell count of the standard cow (222 000/ml) and presented as the excpected SCC per cow. The interaction of parity × stage of lactation × infection status also significantly influenced SCC. On the basis of expected SCC of uninfected cows correction factors were calculated for individual cows with various parities and at various stages of lactation. We conclude that the use of these correction factors can improve the analysis of SCC in the diagnosis of mastitis.

1 Introduction

The regional Animal Health Services and the Central Veterinary Institute have conducted national mastitis surveys in the Netherlands every five years since 1973 to study the prevalence of different forms of mastitis and the effect of mastitis control measures. The prevalence of mastitis has decreased since 1973, probably because of changes in herd management. The surveys have revealed an increase since 1975 in herd size, the use of milking parlours, dry cow treatment (DCT), and disinfection of teats (Table 1) (1-3). One of the purposes of this study was to show the prevalence of mastitis in the Netherlands since 1973.

Both herd and animal factors influence somatic cell count (SCC) (1-10). They cause an effect on SCC which hinders the use of a fixed SCC threshold

Table 1. Percentage of cows in the Netherlands affected by six factors related to herd management from 1975 to 1985.

Factors	% of cows		
	1975	1980	1985
Central transport of milk	42	88	98
Use of milking parlour	unknown	61	79
Dry cow treatment (DCT)	36	57	58
Disinfection of teats	22	41	60
Inspection of milking equipment	46	79	93
Herd size ≥ 50 cows	16	43	56

to differentiate between mastitic and healthy cattle. Statistical analysis was used to determine those factors that have an effect on SCC and to measure this effect. Correction of SCC for these factors — such as parity and stage of lactation — would improve analysis of SCC in the diagnosis of mastitis. Such correction factors were calculated in this study.

2 Materials and methods

A stratified random selection of 227 dairy herds in the 12 provinces of the Netherlands was made based on agricultural data from 1984 collected by the Central Bureau for Statistics in the Hague. The number of cows surveyed was proportional to the total number of cows per province. Herd sizes reflected the regional and national distribution. Quarter milk samples from 10 336 cows were collected once between 1 September 1985 and 1 September 1986 by personnel of the regional Animal Health Services. SCC of quarter milk samples were determined by Coulter Counter according to the methods described by the International Dairy Federation (IDF) (11). Pathogens were isolated and identified according to standard methods (11).

2.1 Mastitis prevalence

To compare the present prevalence of subclinical mastitis with that of earlier surveys, a former IDF definition of mastitis was used: quarter milk was considered mastitic when bacteriologic examination revealed udder pathogens and SCC was ≥ 500 (expressed in thousands/ml). Quarter milk was considered normal when bacteriological examination revealed no udder pathogens and SCC was <500. Data were collected in a data base and processed by the TNO Institute of Applied Computer Science (ITI-TNO). The design of the present survey was similar to that of the previous surveys (1-2); the size was also com-

parable, i.e. 0.4 % of the total number of dairy cattle. This enabled us to the compare the prevalence of bovine mastitis in the Netherlands in 1985-1986 with that in the past.

2.2 Statistical analysis

The statistical analysis was based on a mathematical model. A total of 10 136 cows from 223 farms were tested. Farmers were interviewed regarding management practices, such as the parity, stage of lactation and breed of their cows; and regarding mastitis control measures. Four herds with incomplete data were excluded from this analysis. The SCC per cow was used as dependent variable and was defined as the logarithm of the geometric mean of the SCC of the four quarter milk samples of each cow. The SCC per cow was considered to be the result of fixed and random effects. A threshold value of $P = 0.05$ was used for calculation of significance. Because data were gathered from farms as a whole, and from cows within farms, and because fixed factors relate to either whole farms or to individual cows, we used a so called split-plot model for our analysis. The farms were the whole plots and the individual cows were the subplots within the whole plots. Examples of whole plot factors were herd size, season, and mastitis control measures; examples of subplot factors were parity and stage of lactation. Each of these factors had two or more categories: factor levels. The estimates of the parameters allowed a quantification of the effect of the various factor levels. For this purpose we introduced the notion 'SCC of the standard cow'. For each level, or combination of levels of the various — fixed — factors, the cow SCC had a certain expectation. The geometric mean of the expectations of all possible combinations was termed the SCC of the standard cow. For each level, or combination of levels in case of interaction, one can indicate the deviation from the SCC of the standard cow. The expected mean SCC for the various factor levels was expressed as the antilog of the $^{10}\log$ geometric mean. Correction factors for parity and stage of lactation were calculated as the quotient of the SCC of the standard cow and the expected mean SCC for each parity and stage of lactation.

In the split-plot model only the main effects and the three factor interaction: parity × lactation stage × infection status, were included. The inclusion of more interactions would lead to too many parameters and consequently to estimation problems. Most factors were tested for statistical significance at farm level: breed, season, geographic region, use of milking parlour, presence of pipelines, position of pipelines (high/low), vacuum level of the milking machine, cluster removal (by hand or automatic), regular inspection of milking equipment, herd size, use of teat disinfection, use of dry cow therapy, and

type of housing (cubicle stalls/tied stalls). Some factors were tested for significance at cow level: parity, lactation stage and infection status.

Estimation of the fixed and random effects is a standard technique in case of a balanced design, which means an equal number of observations per combination of factor levels. Since the design of this experiment in this study necessarily was unbalanced, we selected the REML method, (REstricted Maximum Likelyhood), as described by Engel et al. (12) for estimating fixed and random effects. The REML method is described mathematically in the Appendix. The computer program was written in GENSTAT (13) by Van den Bol(14).

3 Results

3.1 Prevalence of mastitis

Between 1973 and 1980 mastitis prevalence declined sharply, but since 1980, udder health has improved only minimally (Fig. 1). In 1973, 69.6 % of all cows were free from mastitis. In 1980, this figure rose to 82.9 % and in 1985 to 84.2 %. The percentage of infected quarters decreased from 16.9 % in 1973 to 9.6 % in 1980 (Table 2). Since 1980, however, the percentage of infected quarters rose slightly to 10.0 %. The frequency of the four most predominant udder pathogens has generally declined since 1973. In 1973, the most frequently isolated pathogen was *Streptococcus agalactiae*, but since then the isolation rate of this udder pathogen has decreased considerably. *Staphylococcus aureus* is now the most frequently isolated pathogen (3.7 %). The four most predominant udder pathogens have a different effect on SCC. This appears in the distribution of SCC of quarter samples from which these

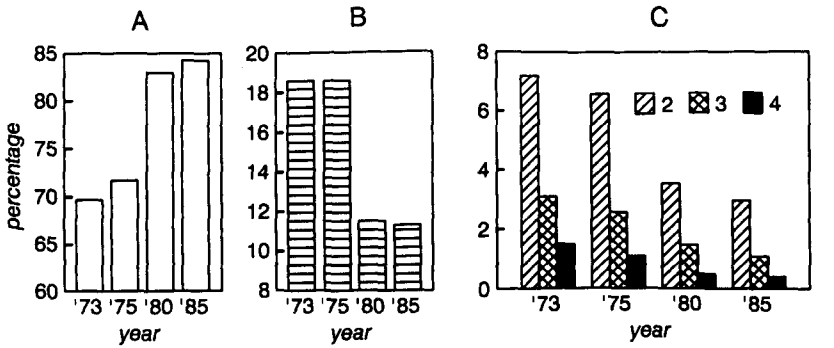

Fig. 1. Percentage of cows with no mastitic quarter (A), one mastitic quarter (B), and two or more mastitic quarters (C).

Table 2. Percentage of mastitis pathogens isolated from quarter milk from 1973 to 1985.

	% of isolated pathogens						
	S.agal.	*S.dysg.*	*S.uber.*	*St.Aur.*	Comb.[a]	Others[b]	Total
1985	1.6	0.9	1.7	3.7	0.3	1.9	10.0 (n=41344)
1980	2.5	1.4	1.4	3.4	0.4	0.6	9.6 (n=33240)
1975	5.4	1.5	1.2	6.1	0.4	0.6	15.4 (n=45012)
1973	6.9	0.9	1.2	6.2	0.4	1.3	16.9 (n=33351)

[a] Comb. = Combination of two or more species *S.agalactiae*, *S.dysgalactiae*, *S.uberis* and *St.aureus*.
[b] Other = Other mastitis pathogens than mentioned under [a].

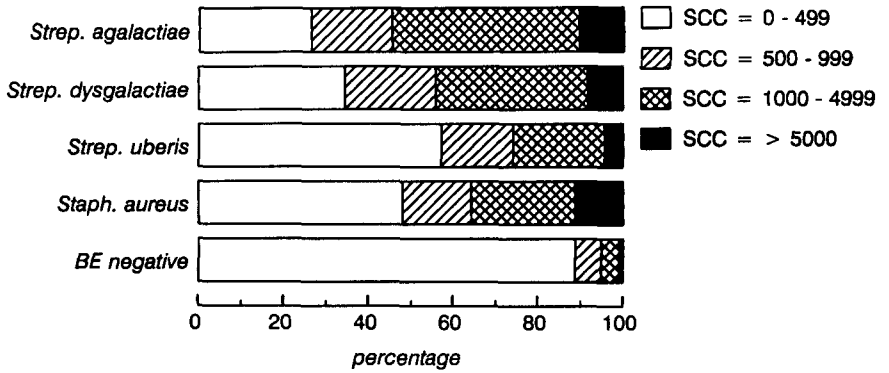

Fig. 2. Distribution of Somatic Cell Counts in uninfected and infected quartermilk. BE = bacteriological examination.

bacteria were isolated (Fig. 2). Apparently *S.agalactiae* more often causes SCC to increase than *S.uberis* or *St.aureus*. More than half (52.1 %) of the *St.aureus* strains were isolated from quarters with a SCC < 500.

3.2 Statistical analysis

Random effects. Variance between herds was 0.027; variance between cows within herds was 0.138; that is the latter variance was 5 times greater than the former variance.

Fixed effects. Breed, season, geographic region, teat disinfection, and the interaction parity × lactation stage × infection status significantly (P=0.000) affected SCC; type of housing was also significant (P=0.047) (Table 3).

Table 3. Factors that significantly affect cow SCC.

Significant[a] factor	Categories of factors (factor levels)					
	1	2	3	4	5	6
Parity	1	2	3	4	5-7	≥8
Stage of lactation	0-1 m[b]	2-4 m	5-8 m	≥9m		
Infection status	-	+				
Breed[c]	FH/HF	MRY	mixed/ other			
Season	Sept-Nov	Dec-Feb	Mar-May	June-Aug		
Geographic region[d]	Gr-Frl-Dr	Ov-Gld	Utr-NH-ZH	Z-Nbr-L		
Teat disinfection	none	selective	all			
Type of housing	cubicle stall	tied stall				

[a] Threshold value for significance: $P = 0.05$.
[b] m = month.
[c] FH/HF = Dutch Friesian/Holstein Friesian breed (black and white); MRY = Meuse Rhine Yssel breed (red and white).
[d] Gr = Groningen; Frl = Friesland; Dr = Drente; Ov = Overijssel; Gld = Gelderland; Utr = Utrecht; NH = Noord Holland; ZH = Zuid Holland; Z = Zeeland; Nbr = Noord Brabant; L = Limburg.

Table 4. Expected mean SCC per cow for five significant factors.

Factor	Expected mean SCC per cow for categories [a]			
	1	2	3	4
Breed	190	264	220	
Season	249	247	209	189
Geographic region	337	222	206	158
Teat disinfection	255	231	186	
Type of housing	197	251		

[a] Categories of factors are given in Table 3.

The SCC of the standard cow was estimated as 222. All factors that were determined by statistical analysis to be significant, substantially affected SCC (Table 4). The interaction of parity × lactation stage × infection status significantly affected the expected mean SCC. Expected mean SCC of infected cattle was higher for each parity and stage of lactation, than for uninfected cattle, with a similar pattern of SCC for both groups of cattle (Fig.3). Expected mean SCC of uninfected cattle were considered to represent the physiologi-

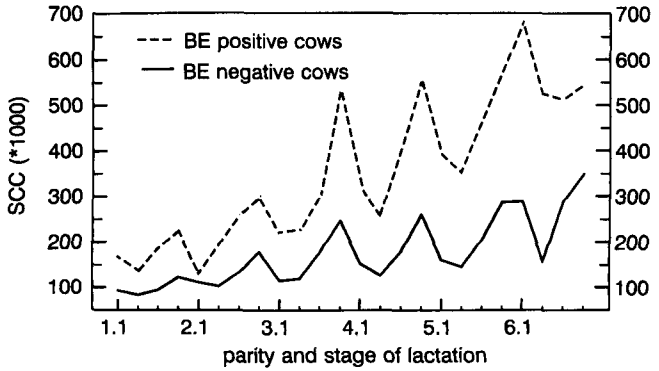

Fig. 3. Expected mean somatic cell count of bacteriological positive and negative cows in relation to parity and stage of lactation. E.g: 1.1 represents first parity, first stage of lactation (months 0-1), 5.3 represents fifth parity, third stage of lactation (months 5-8). BE = bacteriological examination.

Table 5. Correction factors for SCC for each parity and lactation stage of uningected cattle.

Parity	Lactation stage (months)			
	0-1	2-4	5-8	≥9
1	2.39	2.68	2.36	1.82
2	2.00	2.16	1.68	1.25
3	1.93	1.87	1.26	0.91
4	1.46	1.76	1.24	0.86
5-7	1.39	1.53	1.09	0.78
≥8	0.77	1.41	0.77	0.64

cal values. Hence only their correction factors for parity and stage of lactation are given (Table 5).

4 Discussion

Since 1973 the prevalence of subclinical mastitis has decreased strongly: from 30.4 % in 1973 to 15.8 % in 1985 (Fig.1). Since 1975 *St.aureus* has replaced *S.agalactiae* as the main cause of udder infections (3). Because *S.agalactiae* is an obligate udder pathogen, that cannot survive outside the udder, treatment with antibiotics, either during lactation or during the dry period, controls mastitis caused by this bacterium better than it can control mastitis caused by the more ubiquitous cocci. This may explain why in the provinces Noord-

Brabant and Limburg, where *S.agalactiae* frequently caused mastitis in 1975(1), udder health has improved greatly. Nationally, however, the percentage of infected quarters rose slightly from 9.6 % to 10.0 %, indicating that *St.aureus* is more difficult to control than *S.agalactiae*. Quarter milk infected with *St.aureus* or *S.uberis* had a SCC < 500 more often than quarter milk infected with *S.agalactiae* or *S.dysgalactiae* (Fig.2). Thus the first two species caused more infections without inflammation. As mastitis caused by *St.aureus* and *S.uberis* is often undetected, these pathogens can be a major source of infection for other cows.

It is generally recognized that good milking practices reduce the incidence of mastitis. Such practices — regular inspection of milking machine, use of milking parlour, central transport of milk, teat disinfection, and DCT — are often associated with herd size. Because management and milking practices have changed considerably in the Netherlands since 1975 (Table 1), we investigated whether these have had a significant effect on SCC and how much they contributed to variation in cow SCC. Several factors were identified and the deviation from the SCC of the standard cow (222) was calculated for each level of the significant factors.

Our analysis of variance showed that season, geographic region, breed, teat disinfection, and type of housing were significant factors (Table 4). The use of milking parlours was shown to be a significant factor in 1980 (2), but not in this study. During summer grazing of 1975 and 1980, high cell counts (≥ 500) were detected in fewer quarter milk samples than in winter (1,2). Expected mean SCC in this study was highest in autumn (Sept. to Nov. -category 1), decreased in the following seasons, and was lowest in summer (June to Aug -category 4). Wiggans & Shook recently found higher SCC in late summer and early autumn (10).

In the northern provinces (Friesland, Groningen, Drente) the expected mean SCC per cow was much higher (337) than in other provinces. The expected mean SCC in the southern provinces (Noord-Brabant, Limburg, Zeeland) was low (158). Since each geographic region had its own laboratory, differences through variation between laboratories cannot be excluded. This may have contributed to the effect of geographic region on SCC (Table 4). Consistent with other studies that report that MRY cattle have higher SCC than the FH/HF cattle (1, 2, 7), we found an expected mean SCC per cow of 264 for the MRY breed and of 190 for the HF/FH breed. The expected mean SCC of the mixed group (220) was close to the SCC of the standard cow. Dry cow treatment did not appear to affect SCC in our study. It did, however, reduce the percentage of infected quarters: 13.1 % of the quarters were infected on farms where DCT was not used, as opposed to 9.2 % on farms where

it was routinely used on all cows. This is in accordance with previous surveys in the Netherlands (1, 2). Bodoh et al.(5) reported that in herds where DCT was used on selected cows, individual cow SCC were lower than in herds where DCT was used on all cows. Hueston et al. observed that using DCT on all cows in a herd was associated with lower SCC than using it on selected cows only, or not using it at all (15).

In accordance with other studies (1, 2, 5, 15), we found that teat disinfection clearly reduced SCC. Expected mean SCC per cow in herds where the teats of all cows were disinfected was 186, which is 69 points lower than the expected mean SCC per cow in herds where teat disinfection was not used (Table 4).

Cattle housed in cubicle stalls had an expected mean SCC of 197, whereas cattle housed in tied stalls had an expected mean SCC of 251.

Variance of cow SCC within herds was five times larger than variance between herds, which indicates that differences between individual cows have more effect on SCC than farm environment (2, 9, 16). Production levels were not included in our data. Although production level and cow SCC are closely related (8, 9, 17, 18), it does not imply a causal relationship. Both low production and high SCC, however, are often caused by mastitis. Therefore, correction for one of both the parameters, while measuring the other, means correction for a parameter one wants to measure. False negative results are possible when high cell counts of mastitis cases are corrected for low production figures.

Parity and stage of lactation influenced expected mean SCC per cow (Fig.3). In contrast, Jaartsveld et al. did not find stage of lactation to be significant (8). They analysed SCC only at two stages of lactation, <32 days and ≥ 32 days; however, SCC during early and late stage of lactation are high (10, Fig.3). SCC increased less within lactation in first parity than in later parities. The correction factors (Table 5) make it possible to calculate the corrected cell count from the observed SCC. To differentiate between breeds the correction factors can be multiplied by 1.17 (222/190) for the FH/HF breed and by 0.84 (222/264) for the MRY breed. The SCC corrected for breed, parity, and stage of lactation of any lactating cow can now be calculated. For example a MRY cow in sixth parity, in ninth month of lactation (category 4) with an observed count of 400 has a corrected count of $0.84 \times 0.64 \times 400 = 215$. This is below the SCC of the standard cow (222) and may thus be considered normal. An FH/HF cow of the same parity and stage of lactation with an identical observed count of 400, has a corrected count of $1.17 \times 0.64 \times 400 = 300$. This SCC is well above the SCC of the standard cow (222), and may not be considered as normal. Such corrected cell counts for cattle of various breeds, pari-

ties and stages of lactatation may help farmers, veterinarians, and others in the dairy industry to better interpret cow SCC of individual cows.

Appendix

Mathematical description of the REML method

The general form of the model is as follows:

$$Y = X\alpha + Z\,b + e$$

(random variables are in italics)

where *Y* is a vector of N observations, X is the design matrix of both fixed effects at whole-plot level and subplot level, α is a p-dimensional vector of fixed effect parameters, Z is a matrix with zeros and ones to structure the whole-plot error, *b* is a q-dimensional vector of whole-plot errors, *e* is a vector of sub-plot errors.

The REML-solution satisfies the following set of equations:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + \Theta I_q \end{bmatrix} \begin{bmatrix} \hat{\alpha} \\ \hat{b} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix} \quad \text{with } \Theta = \frac{\sigma^2_e}{\sigma^2_b}$$

These equations cannot be solved explicitly, because of the unknown Θ, and have to be solved iteratively. This works as follows:

step 1: obtain initial estimates for σ^2_e and σ^2_b
step 2: solve the REML-equations for $\hat{\alpha}$ and $\hat{b}$:
$\hat{b} = (Z'MZ + \Theta I_q)^{-1} Z'MY$ and
$\hat{\alpha} = (X'X)^{-1} X' (y - Z\hat{b})$
with $M = (I_N - X(X'X)^{-1} X')$
step 3: obtain new estimates for σ^2_e and σ^2_b as follows:
$\sigma^2_e = y'(y - X\hat{\alpha} - Z\hat{b})/(N-p)$
$\sigma^2_b = \hat{b}'\hat{b} / (q - \Theta \text{ trace } (Z'MZ + \Theta I)^{-1})$
Step 4: repeat step 2 and step 3 until convergence is achieved.
References: 1; 3; 6; 16.

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Samenvatting

U. Vecht, H. J. Wisselink en P. R. Defize, *De Landelijke Steekproef Mastitis. Het effect van bedrijfs en dierfactoren op het melkcelgetal*

De doelstelling van de Landelijke Steekproef Mastitis is het verkrijgen van inzicht in de prevalentie en aard van subklinische mastitis bij melkvee in Nederland. Daartoe werd kwartiermelk van 10 336 koeien op 227 bedrijven in Nederland in een gewogen aselecte steekproef in de jaren 1985 - 1986 onderzocht. 84,2 % van de dieren bleken vrij van mastitis. Tien procent van alle kwartieren was geïnfecteerd, waarvan 3,7 % door *Staphylococcus aureus*, die daarmee de belangrijkste uierpathogeen bleek. Met behulp van een 'split-plot' model uit de mathematische statistiek werd het effect van diverse bedrijfs- en diergebonden factoren op het melkcelgetal onderzocht. Een aantal factoren had een significant effect op het celgetal, dit waren ras, seizoen, geografische streek, huisvesting en het gebruik van speendesinfectie. De grootte van deze effecten werd berekend als afwijking van het gemiddelde melkcelgetal van de standaardkoe (222 000/ml). Deze effecten werden weergegeven als het verwachte melkcelgetal per koe. Ook de interactie pariteit × lactatiestadium × infectiestatus van het uier had een significant effect op het melkcelgetal. Op basis van het verwachte melkcelgetal per koe van niet-geïnfecteerde koeien werden correctiefactoren voor pariteit en lactatiestadium berekend. Deze correctiefactoren kunnen een verbetering betekenen voor de interpretatie van het melkcelgetal bij de diagnostiek van mastitis.

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