Alternative Use of Somatic Cell Counts in Genetic Selection for Mastitis Resistance

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Abstract

Various alternatives in analyzing somatic cell counts (SCC) as indirect indicator of resistance of mastitis are discussed by integrating knowledge of the dynamics of the infection and statistical methods used in quantitative genetics and epidemiological research. This is done by (1) defining various measures of SCC, (2) identifying non genetic factors of SCC variation, and (3) proposing a new alternative to analyze the data.

1. Introduction

Several studies have shown variability among cows for natural resistance or susceptibility to udder infections. Spontaneous recovery (self cure) occurs in about 20-50% of confirmed infections. There is considerable variation between individual cows in the percentage of *E. Coli* killed after challenge (Blowey and Edmonson, 1995). Association of mastitis with various MHC alleles have been observed in different cattle populations (Weigel et al., 1990; Lunden et al., 1990; Dietz et al., 1997; Kelm et al., 1997).

Selection for resistance to intramammary infection (IMI) may therefore be possible. However, a first difficulty for researchers in this field has been to define clearly a trait for selection . Mastitis is a complex disease with degrees of severity and various causes. Types of mastitis may be classified on the basis of the severity of the inflammatory response (from peracute to subclinical) and on the basis of mode of transmission (contagious or environmental). A second difficulty is data availability. Indeed, data on clinical cases and intramammary infections are very costly and difficult to acquire. Therefore, many variables have been proposed as indirect indicators of mastitis, such as somatic cell counts (SCC), N-acetyl-β-D-glucosaminidase, bovine serum albumin (BSA), antitrypsin, milk electrical conductivity, and sodium, potassium, and lactose content (review in Detilleux et al., 1997, Annales de Med. vet).

An advantage of SCC over all other indicators is data availability. Indeed there is no nationally organized schemes for recording the other variables, at least in Belgium, while SCC data are often recorded as part of national milk recording. However, problems exist with SCC as an indicator of genetic resistance to mastitis. First of all, genetic correlation between SCC and clinical mastitis is not perfect and averages only .70 (.30 - .89) (Mrode and Swanson, 1996). Reasons for this low estimate of genetic correlation may be attributed in part to inaccurate recording of clinical mastitis. Another problem is the wide range of SCC heritability reported in the literature. In a review of heritability for SCC published in the literature since 1982, Mrode and Swanson (1996) reported estimates from .05 to .19. This wide variation is partly due to differences in models (various fixed effects, test day or lactation average SCC, ...), estimation procedures (REML, Gibbs sampling or Anova-like methods, ...) and in populations used in those studies (sample selected on the basis of another trait, breed, mastitis prevalence, ...). Finally, questions about the importance of somatic cells in milk for protection from invading microorganisms are still unanswered. Milk SCC are constituted of polymorphonuclear neutrophils (PMN) (5-20% in healthy gland) which play an important role in the defense of the gland. Bacteria that pass the teat canal (first line of

defense) enter the teat cistern and meet the second line of defense: the phagocyte leukocytes. Once an inflammatory response has been initiated, neutrophils are the first cells to be recruited to sites of infection. The major role of PMN is to phagocyte and destroy infectious agents. During infection, milk SCC which are consisted of >95% of PMN, are thus indicators of the inflammatory response but they are also the cells necessary to combat the infection. The SCC are indicators of both the resistance and the susceptibility of cows to IMI.

The purpose of this paper is to propose alternative methods for analyzing SCC to better identify animals most resistant to IMI.

2. Material and methods

2.1. The problem

Before choosing an appropriate model to analyze SCC data, it is important to ask ourselves what we want to select for. We want to select for cows resisting best to the entry of pathogens in the gland and for cows able to respond quickly to intramammary infection (IMI), and this in any environment. We want to eliminate cows with long standing chronic infections which cause much tissue infected and scarred, and reduced quality and quantity of milk. Those cows are also long-term carriers able to spread infection to other susceptible cows in the herd.

The problem is thus to find methods to identify cows we want to select for on the basis of the only available variable : their SCC. We'll look at this problem by finding (1) how to use monthly SCC in the left hand side of statistical models, (2) how to define non genetic factors in the right hand side of models, and (3) how to analyze the data. A polygenic genetic mode of inheritance is assumed for SCC so that classical quantitative genetic approach can be used for the genetic part of the model.

2.1.1. Measures for SCC

For individual cows, SCC are generally measured monthly, at the time of milk recording. A log transformation is often applied on counts to reduce skewness and kurtosis of original counts, increase normality, improve additivity and homogenize variance (Ali and Shook, 1980). In most studies, arithmetic mean of log-transformed SCC or individual monthly log-transformed SCC are used.

The lactation average does not take into account the dynamics of the PMN emigration in response to infection. Some cows react rapidly to infection and cure the infection because high number of phagocytically active blood PMN migrate rapidly in milk. Others do not respond as rapidly to the IMI and have moderate to high SCC for long periods. But both type of cows could have same lactation averages (Detilleux et al., 1996). On the other hand, monthly SCC are very variable because of the numerous uncontrollable factors of variation of monthly SCC (stress, presence of other diseases**,** milking frequency, within and between day variation, number of quarters infected, ..). There is a constant interaction between pathogens and defense : Multiplication of pathogens is followed by an augmentation of milk PMN, resulting in a reduction of the number of pathogens and by feedback to a reduction of number of milk PMN. If some bacteria survive, they multiply again as soon as the leukocytic barrier is decreased. This cycle may occur anytime between 2 consecutive monthly milk samplings.

Besides lactation averages and test day SCC, various other ways of using SCC may be proposed for udder health surveillance. Examples are (1) proportion of test day SCC above or below a given limit, (2) direction (increase/decrease) and speed (rapid, gradual, none) of change in test day SCC, (3) time until SCC reach a given limit, (4) difference between observed SCC and SCC expected under healthy conditions, (5) area under SCC curve, and (6) rolling averages. Depending upon the goal of the study (estimation of IMI prevalence, IMI incidence, risk of IMI, ...), one of those variables can be used. Rolling averages are often used for epidemiological surveillance in field studies; area under curve and SCC changes may be used to describe trend over some time period; and time until SCC exceed a given threshold may be used to measure immune response to infection. Understanding SCC sensitivity and specificity is important when choosing a SCC threshold for diagnosing IMI. Sensitivity is the ability of SCC to accurately detect IMI and specificity is the ability of SCC to detect non infected cows. In a Belgian study (referred as study Q, for 'Quarter SCC', in the text), a total of 12,281 quarter milk SCC was collected from 2,332 cows in 154 volunteer herds

surveyed between January 1983 and January 1989 by the Milk Committee in the Province of Luxembourg (Arendt et al.,1997). In this study Q, a limit of 100,000 cells/ml was found for which sensitivity and specificity were equally close to 60%. This means that 40% of quarter SCC >100,000 were not infected and that 40% of quarter SCC <100,000 were infected. Our conclusion is that SCC were not very effective in diagnosing mastitis in our study population. Fuzzy logic methodologies could be applied to this problem (Orlovski, personal communication).

2.1.2. Non genetic factors of variation

To compare cows for their genetic ability to resist IMI, we need to define what non genetic factors are important source of variation of SCC and to include them in our statistical models so that cows may be compared as if they have had the same opportunity to become infected. Factors that influence both healthy and infected cows are physiological SCC variation, stage of lactation, and season.

Physiologic SCC variation, linked to mammary function, includes variation with milk fractions (foremilk, stripping milk, residual milk), and the variation within and between days. The normal sterile gland is supplied with a constant source of PMN migrating from blood as a result of nursing or milking (reviewed by Paape and Capuco, 1997) and this may explain some variation linked to milking frequency. In a better world, SCC sampling hours should be kept constant and milking should be at the same frequency. But as the pessimist says, this is the best world.

The SCC vary also with the stage of lactation: the SCC curve is inverted to the curve for milk production. In healthy cows, this variation is related only with the variation in the quantity of milk produced at different stages of lactation. We estimated this so-called 'dilution effect' on 109,945 monthly SCC on 5,634 cows registered in the national recording system in the Northern part of Belgium between January 1992 and December 1995 (Study C, for 'Cow SCC', in the text). Only SCC obtained before the 600th day of lactation, on cows in 1st three lactations, calving between 500 and 3,000 days old were used in the analysis. The linear decrease in monthly $\log_e$(SCC/1,000) per kg increase in monthly milk yield was estimated at -.0426 (-.0420 to - .0435) per kg milk in 1st parity cows having all monthly SCC <200,000 and by -.0525 (-.0518 to -.0531) in 2nd and 3rd parity cows having all test day SCC <200,000 (Figure 1; Detilleux et al., 1997a). Stage of lactation is also a factor of variation for the risk of infection. In milking cows, most environmental IMI occur during early lactation when cows are metabolically stressed (and this stress may result in clinical outbreaks of subclinical infections acquired during dry period or at calving time. On the other hand, prevalence of *S. aureus* drops immediately after calving, remains constant in heifers and rose in multiparous cows from week 1 to week 3 (Matthews et al., 1992).

Another source of variation in SCC often observed in cows free of IMI is the seasonal variation. This may be related with the distribution of calving throughout the year. In study Q, quarter SCC were analyzed by least squares means techniques with a linear model including fixed effects for herd milking type, herd sanitary status, herd sampling size, year and season of sampling, breed, quarter position and type of organisms found in the gland. Herd effect was assumed normally distributed N (0, σ^2_h) and used as experimental error for testing the significance of the fixed herd effects. The residual error was assumed normally distributed N (0, σ^2_e) and was used to test the significance of the other fixed effects. Quarter SCC were significantly ($p<.001$) lowest from December to May, when most cows are in early lactation. Compared to SCC obtained between June and November, least squares solutions for $\log_{10}$(SCC/1000) were .12 (se = .014) lowest for non infected quarters. On the contrary, others observed in 208 cows (404 lactations) free of symptoms of clinical mastitis a significant increase in individual SCC from May to September (Coulon et al., 1996). Different seasonal pattern of IMI have also been observed. Calvings in July and August were associated with increased odds (1.45 - 1.6) of clinical mastitis in 1st parity Swedish Friesian cows (Oltenacu P.A. and Ekesbo I., 1994).

Although parity is not an important factor of SCC variation in non infected cows (Kirk, J.H. 1984; Paape et al., 1997), there exists variation between parities in infected cows because opportunities of becoming infected increase with age, because udder of older cows have been exposed to many aggressions in previous lactations allowing easier access for bacteria to mammary gland, and because immune response of older cows may be not as effective in bacterial elimination as

response from younger cows. Increase with parity number in cow SCC have been observed in study C (Figure 2).

In infected cows, SCC varies also with the dynamics of the infection. Whether or not IMI occurs and which type of mastitis takes place, depends of the interaction between host, agent, and environmental factors. Environmental determinants and zootechnic factors of the occurrence and outcome of IMI are the design and function of milking machine, milking hygiene practices, level and quality of alimentation, type of housing and weather. Prophylactic measures of mastitis control such as dry cow therapy, post milking teat disinfection, use of intramammary devices, improvement of milking procedures reduced not only the prevalence of major pathogens but altered also the distribution of mastitis pathogens in clinical cases. In high bulk milk SCC herds, *S. agalactiae* and *S. aureus* are the predominant pathogens, whereas in low bulk milk SCC (annual average <150,000 cells/ml) infections with *E. Coli* and environmental Streptococci were most prevalent (Schukken et al., 1990; Hillerton et al., 1995). Deficiency in alimentary selenium and high herd milk production have also associated with high rate of mastitis (Blowey and Edmonson, 1995). Effects on SCC of all environmental determinants and zootechnic factors are regrouped in the so-called 'management group'. In Study C, cow $\log_e$(SCC/1000) were pre-analyzed by least squares means techniques with a linear model including fixed effects for herd*year*season of test, breed, month in milk, milk, parity, age at calving, previous lactation average and average herd milk productivity. The residual error was assumed normally distributed $N(0, \sigma^2_e)$ and was used to test the significance of fixed effects. This preparatory model explained 39.11% of total variation in cow $\log_e$(SCC/1000), from which herd*year*season of test explained as much as 90.31%.

The herd level of infection is another significant characteristics of dynamics of infection. In similar management groups, level of infection may be different so the risk for a cow to become infected can be unlike. Available indirect indicators of the level of infection at herd level are tank cell counts or the percentage of cows infected in the herd. High tank cell counts reflect a high current prevalence of infections of long duration in the herd. Tank cell counts (TCC) are also influenced by the size (number of cows), the structure (number of cows per parity and for different stages of lactation), the level of productivity (dilution effect and genetic correlation), and the quota management (exclusion or not of milk from diseased cows) of the herd. To overcome these problems and because tank cell counts were not available in study C, we defined the infection pressure as the percentage of cows with all monthly SCC<200,000 over entire lactation. Herds were classified as 'healthy' if >80% of cows had all monthly SCC<200,000 over a period of 6 months. All other were defined as 'contaminated'. Average $\log_e$(SCC/1000) were 4.11 (SE = .006) and 4.72 (SE = .007) for healthy and contaminated herds, respectively.

The predominant pathogens species to which cows are exposed is another factor influencing cow SCC. If SCC range from 40,000 to 80,000 cells/ml in cows free from IMI (Paape et al.,1995), SCC may increase over 10^8 within hours after infection. Organisms infectivity and pathogenicity influence the immune response and thus the SCC. Contagious pathogens such as *S. aureus* and *S. agalactiae* tend to cause more sustained increases in SCC than *E. Coli* that usually causes immediate increases in SCC that may quickly decrease (Blowey and Edmonson, 1995). In study Q, least squares solutions were obtained for the effect of bacteriological status on SCS. Compared with quarters infected with coagulase negative Staphylococci, $\log_{10}$(SCC/1000) were greater by .57, .28, and .48 for *S. agalactiae*, for coagulase positive Staphylococci, and for all other mastitis pathogens, respectively (Figure 3). Epidemiologic keys have been proposed to identify most prevalent pathogens, as shown in Table 1 (adapted from Serieys, 1991). When frequency of clinical cases is unknown, cows may be classified as non infected if all consecutive SCC are <300,000 cell/ml over the entire lactation period, as chronically infected if 2 or more consecutive SCC >800,000 cell/ml, and as suspicious in all other cases (Berthelot and Bergonier, 1996). Furthermore, identification of most prevalent pathogens is important because mechanisms of defense against infection by contagious and environmental pathogens may differ (Paape et al ., 1995). Teat shape (diameter and length), streak canal (teat sphincter, keratin, canal diameter and length, Furstenberg's rosette) play important role in resisting pathogens entry into the gland (Miller et al., 1992; Capuco et al., 1992). In this context, different udder conformation traits could be used as indicators of the cow's ability to resist pathogens

entry in the gland at milking time (teat ends conformation not adapted to machine milking) or from pathogens in the environment (teats and udder more susceptible to trauma and environmental colonization). In theory, no increase in milk SCC should be observed if pathogens remain in teat duct (teat duct colonization), as opposed to true udder infection. Once penetration of pathogens in the udder has occurred, neutrophils emigrated from the blood compartment. In 10 Holstein cows experimentally infected with *S. aureus*, Daley et al. (1991) observed cyclic increases and decreases in the quantity of viable bacteria shed in the milk, a concomitant inverse cycling in the number of milk SCC, and a strong relation between PMN number and their phagocytic ability to kill intracellular *S. aureus* varied also in a cyclic manner. The various virulent factors (alpha and beta toxins, coagulase, microabscesses, protein A) of *S. aureus* allows it to survive phagocytosis by PMNs (Blowey and Edmonson, 1995). Endotoxins released by *E. Coli* initiate local inflammatory response. Pre-infection chemotactic response of blood PMN and number of blood PMN before challenge showed a negative correlation with *E. Coli* growth in infected quarters after challenge and with severity of induced coliform mastitis, respectively (Heyneman et al., 1990; Kremer et al., 1993; VandePutte-Van Messon et al., 1993). The efficacy of the aerobic phagocytosis of blood PMN before experimental infection with *E. Coli* is inversely associated with the magnitude of milk losses after infection (Heyneman et al., 1990; Zecchoni et al., 1994).

2.1.3. Models for analyzing SCC

In a preparatory analysis of data collected in study C, animal models were applied to data: $y = X\gamma + Z\alpha + e$, where y = vector of observations, γ = vector of fixed effects, α = vector of random effects, and e = residual error. The vector y was assumed $N(X\gamma, V)$. Fortran packages from Misztal (1989) were used to obtain genetic parameters. Separate analyses were done for :

(1) monthly $\log_e(SCC/1000)$; γ = management group, herd productivity level and sanitary status, month in milk, age at calving, and monthly milk; α = additive genetic value and permanent environmental effect,

(2) $\log_e$ of lactation average

$$\frac{\sum_{lactation} SCC * milk}{\sum_{lactation} milk}$$

where

SCC and milk are monthly data

γ = management group, herd productivity level, length of lactation and age at calving

α = additive genetic value and permanent environmental effect,

(3) proportion of $\log_e(SCC/1000)$ above 200,000 over part of lactation; γ = management group, herd sanitary level, length of period, month on milk at start of period, age at calving, and monthly milk; α = additive genetic value and permanent environmental effect.

Results of these analyses are shown in Table 2. Heritability estimates are consistent with those published in the literature (Mrode and Swanson, 1996). Heritability estimates decreased with increasing lactation number as reported by Banos and Shook (1990). Our model, however, is still rudimentary. Among other imperfections, it didn't account for the correlation between successive lactation and between successive records within one lactation. Some autocorrelation structure could be adopted to model these autocorrelations. Also, we applied the same model to infected and healthy cows, because their health status were unknown. A more attractive statistical approach to analyze these data is the linear finite mixture model.

Finite mixture models are used when population under study is believed to be composed of distinct number of subpopulations. And this is the case for the distribution of SCC among infected and healthy cows. Looking at frequency distribution of quarter SCC in infected and non infected cows from study Q (Figure 4), we discovered 2 overlapping normal distributions with different means but similar variance.

For analyzing SCC, finite mixture densities may be defined as follows: Let $p_d = Pr(Y = y_i \mid D = d)$ be the probability for cow i to have $SCS = y_i$ given D = d (d = 0, 1).

Then, the probability that i^{th} cow will have $SCS = y_i$ is given by :

$$g(yi) = \sum_{d=0}^{1} \pi d\, gd(yi),$$

where

$\pi d = Pr\,(D = d)$ and $gd\,(yi) = Pr(yi\_D = d)$.

This equation is a finite mixture model with two mixing proportions $\pi 0 \wedge \pi 1$, and two component distributions $g0(yi) \wedge g1\,(yi)$.

The posterior probability for cow i with SCS = y_i to be D = d is given by :

$$\tau di = \frac{\pi d\ gd(yi)}{\pi 0\ gd(yi) + \pi 1\ gd(yi)},$$

with

τdi = Pr(D = d| Y = y_i). A cow with Y = y_i and unknown IMI status can be assigned to the group to which it has the highest posterior probability of belonging by the Bayes' rule of association which maximizes the overall allocation rate (Everitt and Hand, 1981).

For SCC data, it may be assumed that D is a discrete random variable that takes the value 0 with probability π_0 , and 1 with probability π_1 . Then E(D) = π_1 and var(D) = π_0 π_1 . The SCS effect y is normally distributed N($X\beta_d$, V), with same V in both component distributions .

The likelihood of this model for all cows is given by :

$$L = \sum_{d=0}^{1} \pi d\ gd(y)$$

where

$$gd(y) = \frac{1}{2\pi^{.5Nd}} \frac{1}{|V|^{.5}} \exp(-.5\,(y - X\beta d)' V^{-1} (y - X\beta d)$$

.

Let $\theta = [\pi 1, \beta 0, \beta 1, V]$, then , the likelihood equations are : 0 =

$$0 = \sum_{d=0}^{1} \tau d\, \frac{\partial \log(\pi d)}{\partial \theta} + \sum_{i=1}^{N} \sum_{d=0}^{1} \tau d\, \frac{\partial \log(g^d(y))}{\partial \theta}$$

Because the first term of the likelihood equation is a function of the prevalence of the disease only, and the second term is a function of the parameters of the component distributions only, the likelihood equations may be split into 2 equations (Jansen, 1993). Maximum likelihood in a generalized linear finite mixture model by using the EM algorithm):

$$0 = \sum_{d=0}^{1} \tau d\, \frac{\partial \log(\pi d)}{\partial \pi 1}, \text{ and } 0 = \sum_{d=0}^{1} \tau d\, \frac{\partial \log(gd(y)}{\partial \beta \partial V}.$$

The maximum likelihood estimates of the parameters can be obtained using the EM algorithm. In the E step, the posterior probabilities τd are evaluated given the current parameter estimates. In the M step, likelihood equations are solved by fixing τd (Jansen, 1993). Then we go back to update the posterior probabilities and the EM cycle is repeated until convergence is reached.

The application of this methodology to data collected in study C is currently under study.

3. Conclusions

Analyses of SCC as candidate for selection against mastitis resistance may be improved by choosing better measures of SCC, by including all non genetic factor of SCC variation in the statistical model and by using methods of genetic epidemiology.

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Table 1. Indicators of most prevalent infections in the herd.

Somatic cell counts	Mastitis clinical cases	Most prevalent pathogens
TCC[1] >500,000 or ICC[2] >40%	K [3]<20%	contagious
TCC <300,000 or ICC <20%	K>40%	environmental

[1]TCC = annual average of herd cell counts
[2]ICC = annual % of individual cell counts >300,000
[3]K = annual % of clinical cases

Table 2. Heritability estimates for somatic cell counts in the Northern part of Belgium.

	N cows with records	N animals in pedigree	Heritability
$\log_e$(weighted lactation average)	4247	76742	.10
Monthly $\log_e$(SCC/1000)	4247	76742	.08
Proportion of $\log_e$(SCC/1000) above 200,000 :			
in 1st lactation	4045	76665	.14
in 2nd lactation	3337	76050	.07
in 3rd lactation	2843	75722	.05

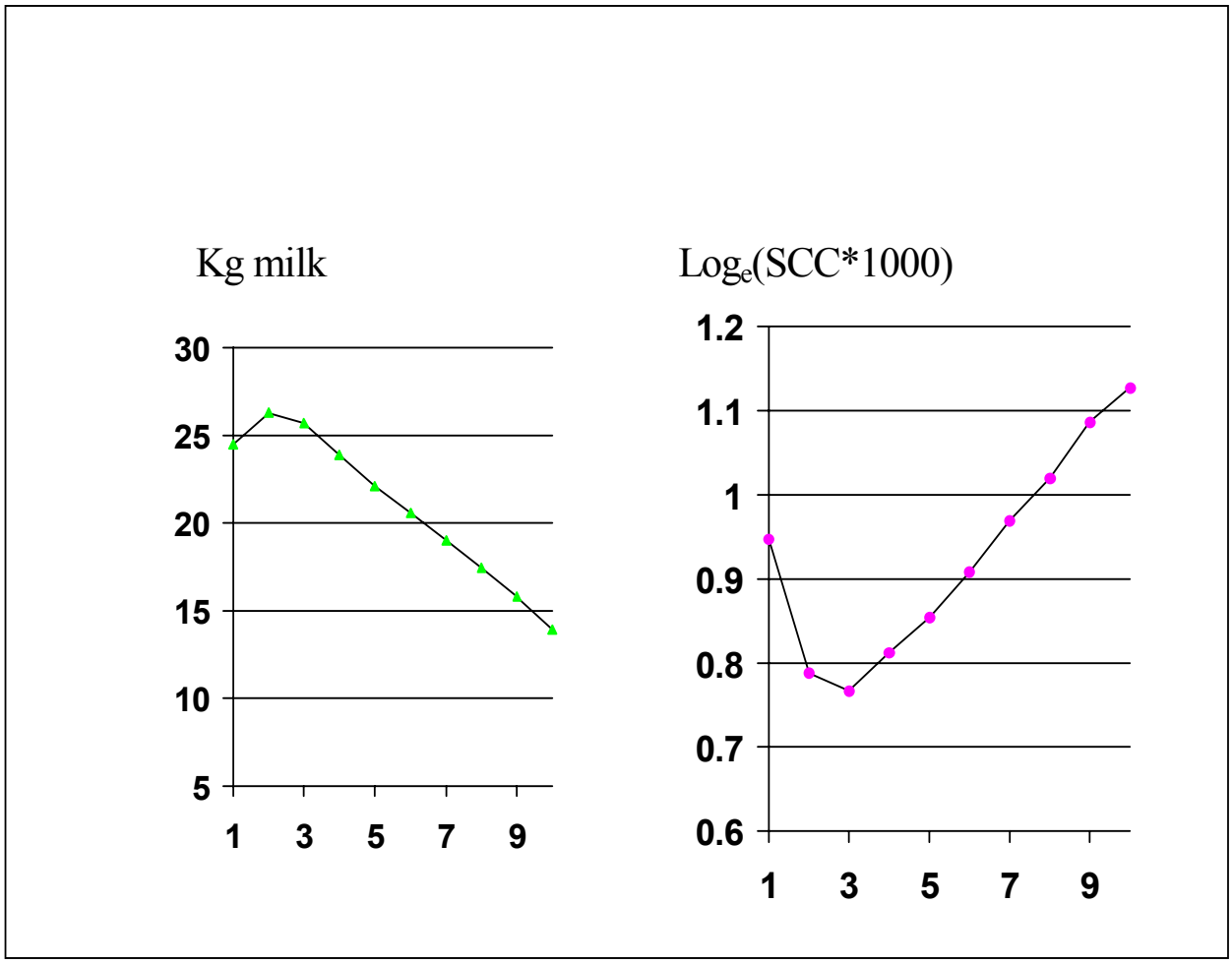

Figure 1. Average monthly milk (Kg) and monthly log$_e$(SCC/1000) in 1st parity Holsteins cows.Figure 2.

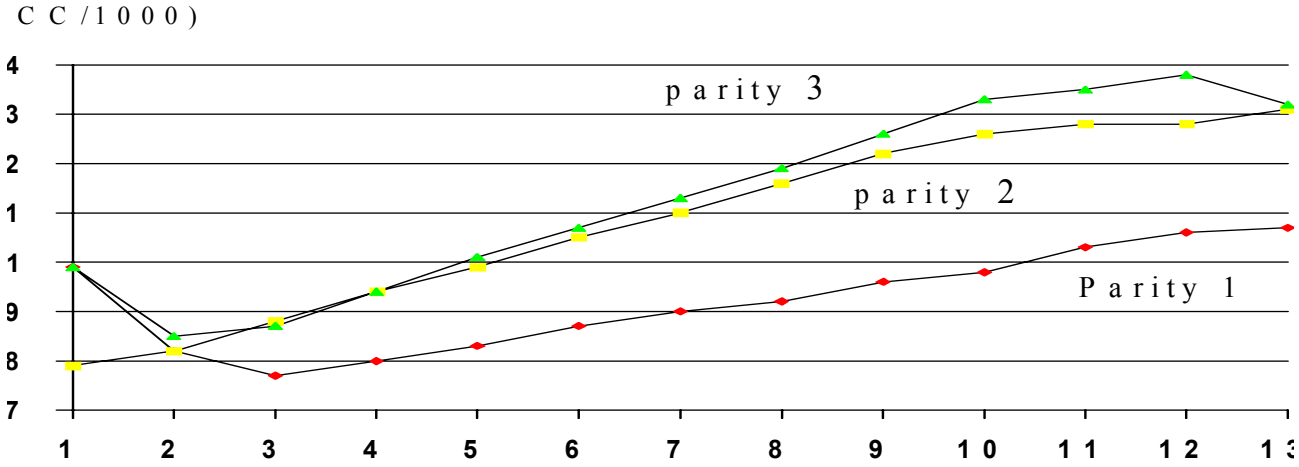

Figure 2. Effect of parity and month in milk on monthly log$_e$(SCC/1000).

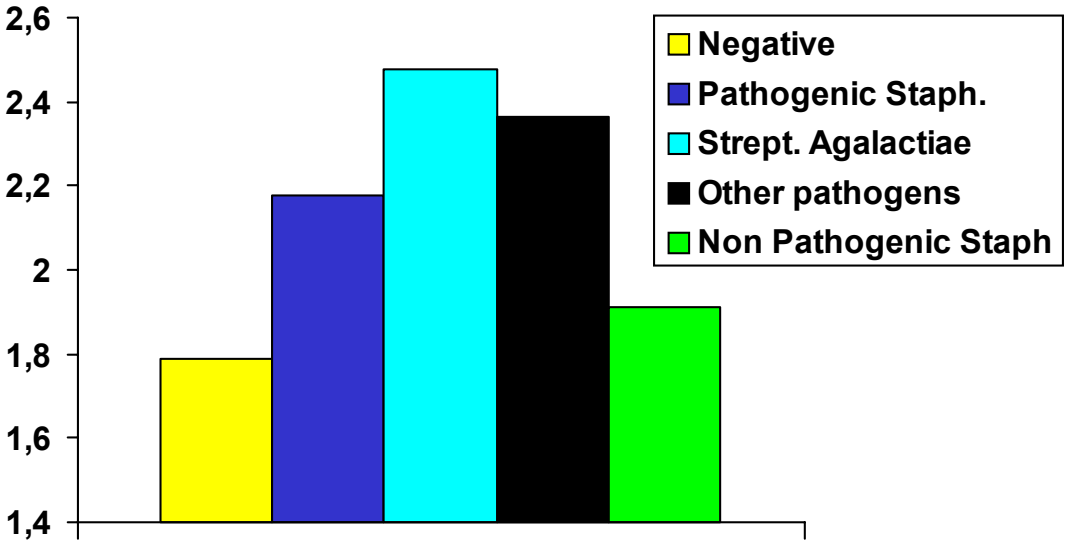

Figure 3. Least squares solutions for quarter $\log_{10}$(SCC/1000) obtained for various bacteria species.

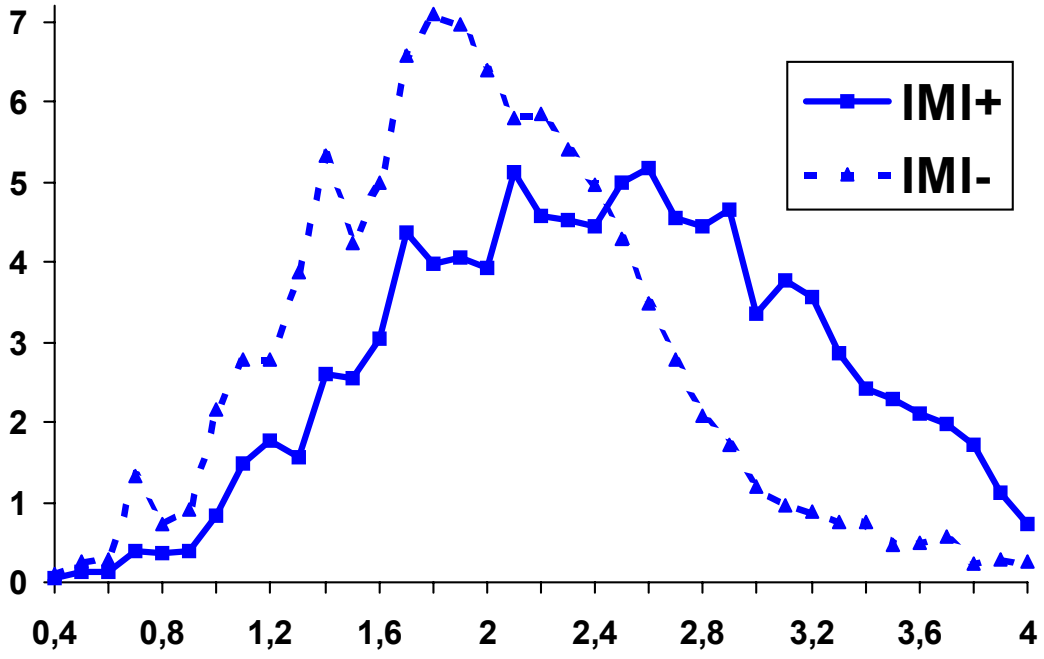

Figure 4. Frequency distribution of quarter $\log_e$(SCC/1000) per mastitis infection status.