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Genetic evaluation of udder health traits in Spanish Holstein cows

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ABSTRACT

The aims of this study were to estimate the genetic parameters of clinical mastitis (CM) and SCS traits, and to compare the performance of genetic evaluations of CM traits using univariate and bivariate analyses (CM-SCS). Data were edited according to the Udder Health Golden Standard harmonization, and then 6 CM traits and 6 SCS traits were considered, as the result of combining 3 lactation classifications (1, 2, ≥3) and 2 milking periods (early, late). The linear mixed animal models included the ratio of period at risk as a covariate, herd-year of calving, month of calving, and lactation-age as fixed effects, and the permanent environmental effect for traits of ≥3 lactations. Prevalence of CM in early lactation was similar regardless the lactation number (5%–6%), and the estimated heritabilities were 0.01. Prevalences in late lactation ranged from 10% to 24% and heritabilities ranged from 0.03 to 0.05. Estimated heritabilities of SCS ranged from 0.06 to 0.16 with univariate analyses. Somatic cell count (therefore its log-transformation SCS) showed a higher probability of correctly identifying healthy cows than infected cows but there was still up to 36% of healthy cows for which CM was not detected by SCS. Genetic correlations between CM-SCS traits ranged from 0.36 to 0.95, and SCS in lactation 3 and later did not add extra information to SCS in the second lactation for predicting CM. Regarding reliabilities of EBVs for CM traits, bivariate CM-SCS analyses led to substantial increases with respect to the single-trait model for sires (7%–12% more in first lactation and 16%–28% more for second lactation). Sire's rank correlations for CM between univariate and bivariate analyses (0.47–0.92) suggest that discarding sires could be more accurate than selecting candidates for sires of dams. We can conclude that SCS in first lactation could be useful to supplement CM data in first and second lactations to improve udder health genetic evaluation.

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INTRODUCTION

Mastitis is one of the most important health problems on dairy farms and, given the nature of milk production, it is impossible to avoid it completely. Farmers are aware of the high economic losses due to the cost associated with treatment of the disease and the detriment of milk composition, with a decrease of the production of CN and lactose and a decrease in fat quality (Harmon, 1994; Nielsen, 2009) and the subsequent damage on animal welfare. Therefore, jointly with the worldwide policies in reducing the use of antibiotics, breeding programs have focused in the past years on selecting animals genetically less susceptible to udder health problems.

Direct selection for mastitis resistance has been difficult because clinical mastitis (**CM**) is not routinely recorded in most countries. Nordic countries started gathering CM information in the 1970s, and the rest of countries started 10 or 20 years later (Zavadilová et al., 2021). In consequence, the first breeding program including selection for resistance to CM was the Norwegians in 1978, and the first international (nondomestic) genetic evaluations of bulls were published by Interbull from 2001 to 2009 (Interbull, 2001; Interbull, 2009). In addition, regardless of the trait definition (i.e., CM as a binary trait or as the number of CM events per lactation) and the statistical model used (lineal model or threshold model), heritability estimates are low, ranging from 0.01 to 0.13 (Pérez-Cabal et al., 2009; Vazquez et al., 2009; Zavadilová et al., 2021). Mastitis is a multifactorial disease influenced by management systems, housing, rearing period, calving stress, dry period, lactation stage, age, number of lactations, and genetic background, among others (Bludau et al., 2016). Several studies have shown that the frequency of CM is higher at the beginning of lactation than in any other period (Emanuelson et al., 1988; Barkema et al., 2006).

Most countries are approaching mastitis resistance selection indirectly using the average SCS per lactation, a

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

log-transformation of SCC that is available from the milk recording system (Schutz, 1994). Somatic cell count is considered an indicator of mastitis and its inflammatory process in the udder because somatic cells include epithelial cells but also leucocytes and macrophages defending the udder against infections (Sharma et al., 2011). There is consensus that the higher SCC, the more likely the presence of mastitis. However, the somatic cells thresholds to consider presence of subclinical mastitis vary in literature. Some authors reported values above 200,000 to 250,000 cells/mL (Schepers et al., 1997; Schwarz et al., 2011; Sadeghi et al., 2021), but higher thresholds, such as 400,000 cells/mL, were set by other researchers (Sears and Heider, 1981; Sears et al., 1991; Polat et al., 2010;). Furthermore, there are different thresholds for primiparous cows than for multiparous cows (i.e., Zecconi et al., 2019; EuroGenomics, 2022a). The estimated genetic correlation between SCS and CM is around 0.7 (Mrode and Swanson, 1996; de Haas et al., 2008; Bloemhof et al., 2009) and the higher heritability of SCS (Carlén et al., 2004) make this trait a key indicator for selection and management of mastitis in dairy herds all around the world. de Haas et al. (2008) have reported that SCC is more informative as an indicator of mastitis because cows in early lactation, during the period from 10 d before calving to 60 d after calving, are more susceptible to mastitis cases than in late lactation. The reasons behind this fact could be stress during calving and pressure on the teat canal, which can increase leakage. This creates a field for invasion of pathogens into the udder of the animal (Singh, 2022). However, Heikkilä et al. (2018) have shown a long pathogen persistence, even a different pathogen pattern for early mastitis cases in Finnish dairy cattle.

Currently, with the development of genomics, EuroGenomics gathers information from 11 European countries to improve Holstein genetics and management. They developed Golden Standards to harmonize data, involving different steps such as the recording practices, testing models and methods, and the genetic evaluation process. Taking into account the higher frequency of CM expected in early lactation, a long pathogen persistence, and even a different pathogen pattern for early mastitis cases in Finnish dairy cattle (Heikkilä et al., 2018), as well as the fact that acute CM before the peak of lactation triggers a rapid and strong inflammatory reaction, which causes a significant and irreversible damage to the mammary tissue, and a large loss of milk production (Schukken et al., 2009), the Udder Health Golden Standard recommends (EuroGenomics, 2022a) splitting the lactation into 2 periods to define 2 different CM traits within each lactation. Moreover, the prevalence rate of CM increases with the number of lactation and consequently leads to a higher economic loss. Although genetic correlations between CM at different lactations are high, they are not close enough to be considered the same trait (Carlén et al., 2004; Bloemhof et al., 2009; Eding et al., 2009).

Each EuroGenomics country must follow these recommendations to adapt their data to that harmonization. In Spain there has been an official genetic evaluation of the average SCS per lactation since 2003, and it is included in the national selection index ICO with 8% of importance to improve udder health. Spain joined EuroGenomics in 2012 and is working on implementing the genetic evaluation of new functional traits such as mastitis and other diseases or traits related to sustainability. Additionally, the Spanish Holstein Association (**CONAFE**) implemented in 2012 the I-SAP project which focused on claw health (Charfeddine et al., 2013) and later, in 2015, the I-SA project (Charfeddine et al., 2018), an extension with the aim of collecting data not only on claw disorders but also on reproductive, metabolic, udder health, and other diseases, to provide new selection tools on health traits for breeders. Thus, CM has been recorded since then, and CONAFE is implementing the official genetic evaluation of udder health. The first step was to study the most appropriate model for genetic evaluation (Pérez-Cabal et al., 2023) following the EuroGenomics Golden Standard. Therefore, the aims of this study were to (1) estimate the genetic parameters of CM and SCS traits and (2) compare the performance of genetic evaluations of CM traits from univariate and bivariate analyses CM-SCS in the Spanish Holstein population.

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Udder Health Traits

The study was performed for different udder health traits, combining information from the official milking recording system and the I-SA project, provided by CONAFE. The indicator trait for subclinical mastitis was the log-transformation to SCS, applying a base-2 logarithmic function on the SCC obtained from the official milking recording system (Ali and Shook, 1980):

$$SCS = 3 + \log_2\left(\frac{SCC}{100{,}000}\right).$$

The I-SA project provided CM information. Clinical mastitis was either diagnosed by the veterinarian or the breeder, according to the atlas of definition of pathologies developed in the frame of the I-SA project (CONAFE, 2021). The atlas defines that CM should be diagnosed when there is inflammation of the udder tissue, touch sensitivity, and hardening of the udder; when milk presents a visually abnormal appearance: purulent, serous, watery, or bloody from one or more quarters; and if the animal has loss of appetite and reduced activity. Each cow was assigned 0 or 1 depending on the absence or presence, respectively, of at least one CM event within a given period of time. Thus, originally CM was defined as binary trait (0/1; absence or presence).

Data Editing

According to the Udder Health Golden Standard, each lactation was split into 2 milking periods (**MKP**): from 10 d before calving up to 68 DIM (MKP = 1, early lactation) and from 69 d up to 305 DIM (MKP = 2, late lactation). In addition, we considered all traits studied as different in first lactation (**L** = 1), second lactation (L = 2), and third or later lactations (L ≥ 3). Therefore, 6 CM traits and 6 SCS traits were studied after combining lactation number and milking period. Each of the SCS traits was obtained by averaging the SCS test-days included in the respective early or late MKP and within lactation.

Only lactations from 1 to 5 cows that calved from January 2013 to February 2022 were used. Records from herds without regular CM information throughout all those years were discarded. Only the records corresponding to the first CM event per lactation and milking period were included in the study. For example, one cow could be diagnosed with CM for the first lactation and both milking periods, and therefore that cow contributed as infected in CM11 and CM12. As in the national genetic evaluations, each SCS record was adjusted by stage of lactation and month (CONAFE, 2023; Interbull, 2023).

In addition, some data requirements were set to accomplish the EuroGenomics Golden Standard for Udder Health traits. Data editing was performed using R software (R Core Team, 2022):

- Because CM11 (i.e., CM in first lactation and early lactation) is the most critical trait in terms of low mastitis incidence, only herd-year groups with at least 5 records and a minimum prevalence of 0.43% (which was the first percentile of the average prevalence by herd-year) were considered in the analyses.
- Binary traits (i.e., CM) were transformed to Gaussian distributions using Snell scores based on herd-year strata.
- Heterogeneous variance per herd-year was considered for SCS, such that each record was precorrected following the EuroGenomics recommendations (EuroGenomics, 2022b).
- Periods at risk were considered as the standardized ratios of days at risk per milking period to include them in the statistical models. There were 2 risk periods for each of the milking periods considered in the study. The risk period for early lactation lasts from 10 d before calving date to 68 d of lactation (i.e., 79-d length). The risk period for late lactation begins the 69th day of lactation and finishes at 305 d (i.e., 237-d length). For a given cow and lactation, the days at risk in early lactation were calculated as the days from the date of the beginning of that period (i.e., 10 d before calving date) to the earlier of these 2 dates: the date of the last record of that cow or the date when gathering udder health data for the herd was finished. The same was done for late lactation but considering the beginning of this period instead (i.e., 69d of milking). Last, ratios were obtained with respect of the length of each respective risk period and standardized within herd.

The final data set for CM and SCS included 97,979 records from 59,772 cows in 262 herds. The 10-generation pedigree was obtained using the R package *pedigreeTools* (Vazquez et al., 2023) and included 162,904 animals.

Statistical Models

Genetic parameters were estimated using ASReml v. 4 (Gilmour et al., 2021) and ASReml-R 4.2 (Butler et al., 2023) running univariate and bivariate analyses implementing the following animal linear mixed model:

$$y_{ijklmn} = \alpha_0 + ratio_i + hy_j + M_k + LAE_l + id_m + pe_n + \varepsilon_{ijklmn},$$

where y_{ijklmn} is each of the 6 CM traits and (or) 6 SCS traits; α_0 is an effect common to all observations; $ratio_i$ is the covariate standardized ratio of days at risk; hy_j is the fixed effect of the jth herd-year of calving (up to 811 levels); M_k is the fixed effect of the kth month of calving (12 levels); LAE_l is the fixed effect of the lth lactation-age (up to 44 levels); id_m is the additive genetic effect of mth animal (162,904 animals); and pe_n is the permanent environmental effect of the nth cow (59,772 cows) for traits in lactations L ≥ 3.

The joint distribution of random effects included in the model was as follows:

$$\begin{pmatrix} \mathbf{id} \\ \mathbf{pe} \end{pmatrix} \sim N\left[0, \begin{pmatrix} \mathbf{A}\sigma^2_{\mathrm{id}} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}\sigma^2_{\mathrm{pe}} \end{pmatrix}\right],$$

where **id** and **pe** are the vectors of animal and permanent environmental effects, respectively, and σ^2_{id} and σ^2_{pe} are the corresponding variances; **A** is the additive relationship matrix, and $\mathbf{I}$ is an identity matrix of appropriate order. The distribution of model residuals is $\boldsymbol{\varepsilon} \sim N\left(\mathbf{0}, \mathbf{I}\sigma_\varepsilon^2\right)$.

The EBV reliability was calculated as $rel_i = 1 - \frac{\left(s.e._i\right)^2}{\sigma_u^2}$, where rel_i is reliability of animal i; $s.e._i$ is the standard error of EBV of animal i, and σ_u^2 is the additive genetic variance of CM estimated by the corresponding analyses.

A total of 1,380 sires with 10 daughters or more in the dataset were considered to compare sires' ranking with CM univariate and CM-SCS bivariate analyses. In our study 971 sires had 50 daughters or fewer, 208 sires had 50 to 100 daughters, and 201 sires had 100 or more daughters. Ranking comparison was assessed estimating the Spearman's correlation between EBV. Standard errors of correlations were estimated from 1,000 replicates using bootstrap methodology (Efron and Tibshirani, 1986).

RESULTS AND DISCUSSION

Descriptive Statistics

Prevalence of CM in early lactation was similar regardless the lactation number (Table 1). In our study, in the second milking period of the first lactation (CM12) the prevalence doubled that from the first period (CM11); in second lactation it was 3 times higher, and 4 times higher in third and later lactations. Because mastitis prevalence depends on lactation stage, milk production, and farms, there is high variability in literature. Previous studies that split lactations in different periods than we did reported more liability to CM in early lactation (Zwald et al., 2006; Negussie et al., 2008), but they considered early lactation as the first 150 DIM while in our study it was half that time (68 DIM). In those studies, prevalence was higher at the beginning of lactation but also increased at the end, becoming chronic mastitis. Koeck et al. (2010) also found similar prevalences in early lactation (up to 50 DIM). Other studies found prevalences from 14% to 24% for complete lactations (Carlén et al., 2004; Negussie et al., 2008; Zavadilová et al., 2020).

As expected, mean SCS increased with parity number, which is clearer for SCC. Table 2 shows the sensitivity and specificity of SCC to identify infected and healthy cows for CM, considering the EuroGenomics SCC thresholds. In our study, specificity was higher than sensitivity (except for CM32). The sensitivity ranged from 47% to 71%, being higher in the late lactation period for all lactations, which was the opposite of the specificity (64%–82%). Then, SCC showed higher probability of identifying correctly healthy cows rather than infected cows but up to 36% of healthy cows had high SCC. This may be because, after a CM event, a healed cow does not recover her pre-infection SCC level, and a latent subclinical mastitis remains. López-Paredes (2022) studied the sensitivity and specificity of SCC and subclinical mastitis diagnosed by the California test in a primiparous Spanish dairy population. He reported moderate or low sensitivity (47%–79%), like our study, but higher specificity (96%–99%) because he was relating SCC to subclinical mastitis instead of CM. Then, SCC and by extension, its log-transformed SCS, are worldwide indicators of subclinical mastitis because the low rate of false positive test detected due to high specificity.

Estimates of Genetic Parameters

In general, the estimated heritabilities, for both CM and SCS traits, were higher in the second milking period than in early lactation. That may be associated with a higher metabolic resources demand accumulated up to the peak of lactation, with the subsequent increase of metabolic stress. Heritabilities of CM traits estimated from univariate analyses (Table 3) ranged from 0.01 (for all traits in early lactation) to 0.05 (CM32). Bivariate analyses led to similar or slightly higher heritabilities than those from the univariate analyses (Supplemental Table S1; see Notes). Other authors reported similar low

Table 1. Prevalence (%) of CM traits and SCS within lactation and milking period[1]

	L = 1	L = 1	L = 2	L = 2	L ≥ 3	L ≥ 3
Item	MKP = 1	MKP = 2	MKP = 1	MKP = 2	MKP = 1	MKP = 2
CM trait	CM11	CM12	CM21	CM22	CM31	CM32
Records (n)	35,801	30,391	22,558	25,361	30,636	31,794
Prevalence (%)	5	10	5	18	6	24
SCS trait	SCS11	SCS12	SCS21	SCS22	SCS31	SCS32
Records (n)	35,801	35,801	28,262	28,262	33,916	33,916
Mean	6.49	6.10	6.39	6.64	6.98	7.38
SCC[2] ($\times 10^3$ cells/mL)	181.20	158.31	216.90	260.11	362.60	462.72

[1]Lactation (L) = 1, 2, or ≥3; milking period (MKP) 1 = from 10 d before calving up to 68 DIM; MKP 2 = from 69 d up to 305 DIM.

[2]Mean raw SCC before log-transformation and precorrection for heterogeneous variance.

Table 2. Sensitivity (Se) and specificity (Sp) of SCC as indicator of CM traits for the corresponding lactation and milking period, according with EuroGenomics thresholds[1]

Lactation number	CM trait			
First	CM11[2]		CM12	
	Infected (1,790) Se = 59%	Healthy (34,011) Sp = 73%	Infected (3,613) Se = 62%	Healthy (26,778) Sp = 71%
Above SCC threshold (n)	1,048	9,328	2,251	7,899
Below SCC threshold[3] (n)	742	24,683	1,362	18,879
Second	CM21		CM22	
	Infected (1,276) Se = 47%	Healthy (21,282) Sp = 82%	Infected (5,161) Se = 58%	Healthy (20,200) Sp = 78%
Above SCC threshold (n)	605	3,815	2,971	4,456
Below SCC threshold[4] (n)	671	17,467	2,190	15,744
Third or later	CM31		CM32	
	Infected (2,152) Se = 57%	Healthy (28,479) Sp = 72%	Infected (8,237) Se = 71%	Healthy (23,557) Sp = 64%
Above SCC threshold (n)	1,227	8,045	5,812	8,524
Below SCC threshold[4] (n)	930	20,434	2,425	15,033

[1]Total numbers of infected and healthy records are within parentheses.

[2]See definition of traits in Table 1.

[3]SCC threshold: 100,000 cell/mL.

[4]SCC threshold: 200,000 cell/mL.

heritability for CM, ranging from 0.01 to 0.08 (Carlén et al., 2004; Bloemhof et al., 2009; Zavadilová et al., 2021), regardless of whether CM was studied as a different trait by lactation, if the lactation number was included as an effect in the evaluation model, or the analyses used linear or threshold models.

Genetic correlations between both milking periods within lactation were strong for lactation 3 and later, intermediate for lactation 2, and weak for the first lactation. That means that cows susceptible to CM in early lactation would not have to be as prone to CM in late lactation, at least in the first and second lactations, and therefore it should be considered as different traits. When comparing within milking periods, the genetic correlations between lactation ≥ 3 and previous lactations in early lactation were high, being almost uncorrelated for lactations 1 and 2. As expected, genetic correlations for the second milking period were higher for closer lactations, meaning a greater propensity of animals for CM in the subsequent lactation. In literature, genetic correlations between CM in different lactations were moderate-high, around 0.60 to 0.90 (Carlén et al., 2004; Bloemhof et al., 2009; Eding et al., 2009). Zavadilová et al. (2017) studied 3 milking periods and genetic correlations between the first one (100 first DIM) and the following were 0.89 and 0.42, showing a moderate-strong association between periods. Genetic correlations between CM31 and CM21 and CM22 showed that these traits behave the same. Al-

Table 3. Heritabilities (diagonal) and genetic correlations (upper triangle) of CM traits within lactation and milking period[1]

Trait[2]	CM11	CM12	CM21	CM22	CM31	CM32
CM11	0.01 (0.004)	0.13	0.05	0.24	0.85	0.37
CM12	0.21	0.03 (0.007)	0.45	0.95	0.78	0.90
CM21	0.29	0.20	0.01 (0.005)	0.63	0.99	0.74
CM22	0.19	0.05	0.23	0.04 (0.009)	0.99	0.99
CM31	0.41	0.24	0.32	0.17	0.01 (0.003)	0.86
CM32	0.17	0.08	0.20	0.03	0.29	0.05 (0.008)

[1]Standard errors are in the lower triangle for genetic correlations and within parentheses for heritabilities.

[2]See definition of traits in Table 1.

Table 4. Heritabilities (diagonal) and genetic correlations (upper triangle) of SCS traits within lactation and milking period[1]

Trait[2]	SCS11	SCS12	SCS21	SCS22	SCS31	SCS32
SCS11	0.08 (0.01)	0.89	0.93	0.88	0.83	0.78
SCS12	0.03	0.17 (0.01)	0.94	0.96	0.88	0.90
SCS21	0.03	0.02	0.06 (0.01)	0.94	0.99	0.98
SCS22	0.03	0.01	0.02	0.14 (0.01)	0.97	0.99
SCS31	0.05	0.03	0.01	0.01	0.08 (0.01)	0.95
SCS32	0.04	0.02	0.02	0.01	0.02	0.16 (0.01)

[1]Standard errors are in the lower triangle for genetic correlations and within parentheses for heritabilities.

[2]See definition of traits in Table 1.

Table 5. Genetic correlations between CM and SCS traits within lactation and milking period[1]

Trait[2]	SCS11	SCS12	SCS21	SCS22	SCS31	SCS32
CM11	0.51 (0.14)	0.43 (0.13)	0.51 (0.14)	0.50 (0.11)	0.36 (0.15)	0.39 (0.13)
CM12	0.56 (0.10)	0.75 (0.07)	0.85 (0.06)	0.90 (0.04)	0.79 (0.08)	0.82 (0.06)
CM21	0.84 (0.19)	0.71 (0.15)	0.87 (0.17)	0.74 (0.15)	0.87 (0.15)	0.77 (0.15)
CM22	0.67 (0.07)	0.80 (0.04)	0.67 (0.10)	0.82 (0.06)	0.90 (0.04)	0.93 (0.03)
CM31	0.61 (0.29)	0.55 (0.21)	0.95 (0.14)	0.84 (0.11)	0.66 (0.24)	0.69 (0.21)
CM32	0.68 (0.08)	0.75 (0.06)	0.83 (0.07)	0.90 (0.04)	0.83 (0.06)	0.82 (0.05)

[1]Standard errors are within parentheses.

[2]See definition of traits in Table 1.

though the genetic correlation between CM21 and CM32 was lower (the farthest milking periods of that lactations), the information from the second lactation could be enough to predict CM in L ≥ 3 lactations. Given our results, we could suggest that CM traits can be reduced to 5 informative traits: both milking periods in the first and second lactations and total CM in lactation 3 and later (not split into 2 milking periods).

Heritabilities and genetic parameters for SCS of our study are in the low range of those reported in literature (e.g., Carlén et al., 2004; de Haas et al., 2008). Univariate analyses estimated heritabilities from 0.06 to 0.16 (Table 4), which increased when performing the bivariate analyses, from 0.08 to 0.36 (Supplemental Table S2; see Notes). Genetic correlations between SCS traits were high, all above 0.83, regardless milking period or lactation number, and SCS in first lactation could be a good indicator of SCS in later lactations.

Genetic correlations between CM and SCS traits are shown in Table 5. Genetic correlations for the same lactation and milking period (diagonal) ranged from 0.51 to 0.87. The lowest correlations were estimated for CM11, being SCS11 and SCS21 the most correlated with it, that is, SCS for the same milking period and following lactations. However, CM12 showed higher genetic correlations with all SCS traits, which ranged from 0.56 to 0.90. Regarding the following lactation, CM21 was more correlated with SCS traits in early lactation than with SCS traits in late lactation, regardless of the SCS lactation number. Similarly, CM22 showed higher correlations with SCS traits within the same milking period. Finally, CM in third or later lactations presented similar trends in terms of the same period the higher genetic correlation, except with SCS in later lactations, where the highest values were obtained with the opposite milking period. These results, in accordance with literature (Carlén et al., 2004; de Haas et al., 2008; Zavadilová et al., 2017), confirm that SCS is not CM, but the estimated genetic correlations and heritabilities suggest that SCS could serve as a good indicator to select for mastitis resistance. Even more, SCS in lactation 3 and later did not add extra information to SCS in second lactation for predicting CM and could be unnecessary.

Reliability of Clinical Mastitis EBV

Single-trait analyses led to mean reliabilities of EBV for CM traits ranging from 3% to 18% for cows and from 6% to 30% for sires. Early lactation showed the lowest reliabilities regardless the lactation number (Table 6), because those CM traits were the less heritable. Bivariate analyses with SCS traits increased substantially the reliabilities for every CM trait with respect to the corresponding trait from the single-trait model. Note that reliabilities for CM31 increased up to 27 times, because the univariate analyses led to the lowest reliability. The permanent environmental variance for CM31 was almost 5 times higher than the additive variance (Supplemental

Table 6. Overall mean (± SE) reliability (%) of EBV for CM traits estimated from single-trait (CM row) and bivariate analyses with each of SCS traits for cows and sires

Trait[1]		CM11	CM12	CM21	CM22	CM31	CM32
CM	Cows	7 ± 3.7	14 ± 5.3	6 ± 3.2	16 ± 5.7	3 ± 2.2	18 ± 6.7
	Sires	14 ± 7.8	25 ± 11.1	11 ± 6.8	28 ± 12.0	6 ± 4.8	30 ± 13.8
SCS11	Cows	11 ± 4.6	17 ± 6.1	17 ± 5.8	23 ± 6.7	82 ± 0.7	39 ± 5.4
	Sires	21 ± 9.4	30 ± 12.1	31 ± 11.6	40 ± 13.5	84 ± 1.5	52 ± 10.7
SCS12	Cows	13 ± 4.5	22 ± 7.0	18 ± 5.4	30 ± 7.5	75 ± 1.0	35 ± 5.4
	Sires	23 ± 9.4	39 ± 12.9	31 ± 10.2	49 ± 13.9	78 ± 2.0	51 ± 11.9
SCS21	Cows	13 ± 4.8	23 ± 7.2	16 ± 5.3	19 ± 6.6	56 ± 3.7	38 ± 6.0
	Sires	23 ± 9.8	41 ± 14.0	28 ± 11.3	32 ± 12.9	64 ± 7.3	51 ± 12.0
SCS22	Cows	15 ± 5.0	29 ± 8.1	16 ± 5.6	23 ± 7.4	30 ± 6.0	15 ± 7.3
	Sires	26 ± 10.3	49 ± 14.5	29 ± 10.9	39 ± 13.9	43 ± 11.4	36 ± 19.1
SCS31	Cows	11 ± 4.4	21 ± 6.4	18 ± 6.4	28 ± 7.8	10 ± 4.2	22 ± 8.0
	Sires	20 ± 9.0	37 ± 12.8	32 ± 13.0	46 ± 15.8	17 ± 8.9	37 ± 15.5
SCS32	Cows	12 ± 4.5	26 ± 7.1	18 ± 6.0	32 ± 8.9	14 ± 5.1	24 ± 8.4
	Sires	22 ± 9.3	43 ± 13.5	30 ± 12.1	50 ± 16.5	23 ± 10.4	39 ± 15.7

[1]See definition of traits in Table 1.

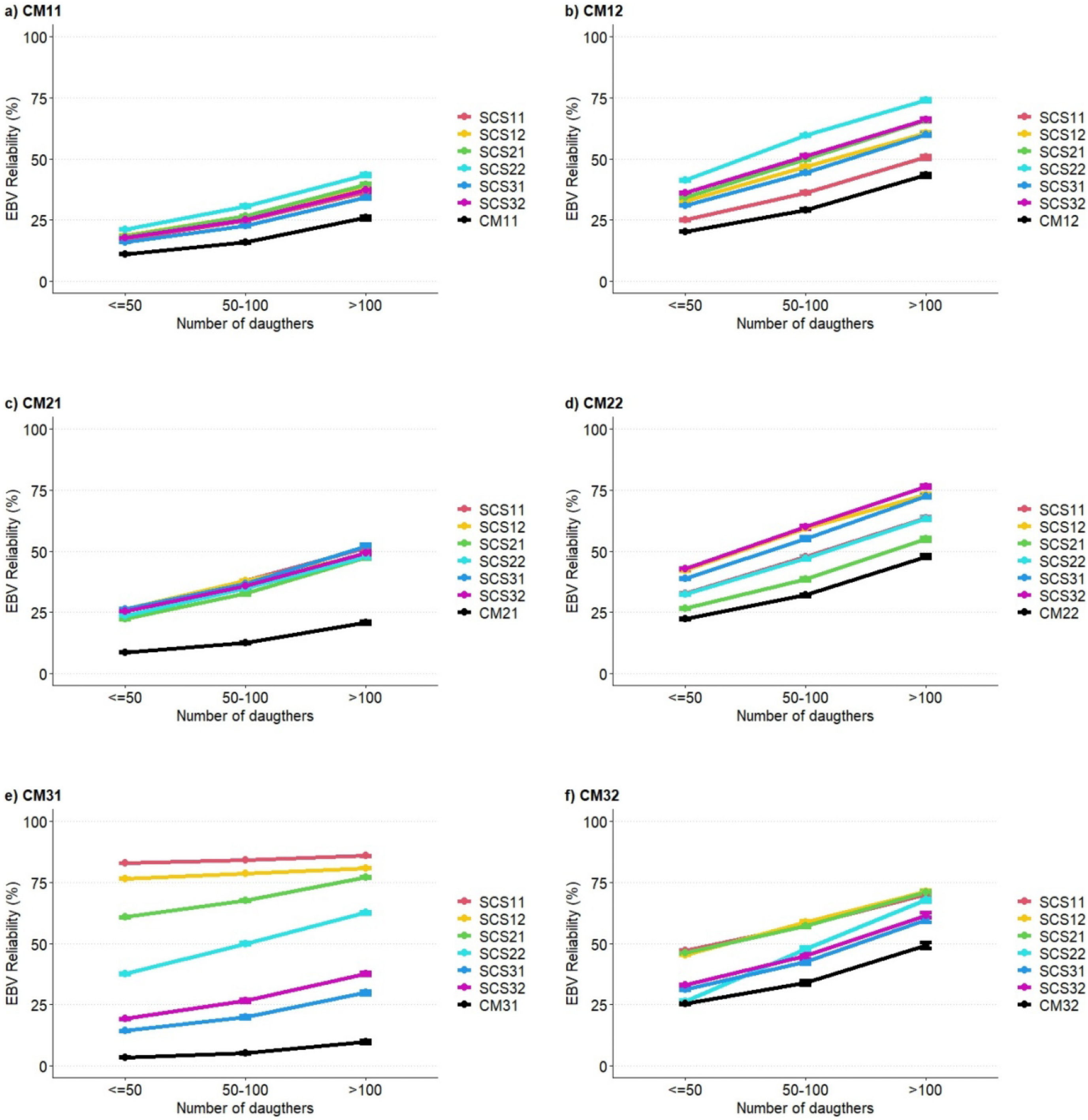

Figure 1. Average reliabilities (%) of EBV of CM traits for sires, grouped by number of daughters, estimated from single-trait analyses (CM) and bivariate analyses (SCS). See definition of traits in Table 1.

Table S3; see Notes), and this additive variance was the lowest among all CM traits. It seemed that SCS information from second lactation helped to improve reliability of EBV in first lactation, and CM in second or later lactations takes advantage of SCS in first lactation. Complementary information about mean EBV is shown in Supplemental Table S4 (see Notes).

Figure 1 shows mean reliabilities of sires by number of daughters. In general, the larger the number of daughters the higher EBV reliability, regardless of the analyses

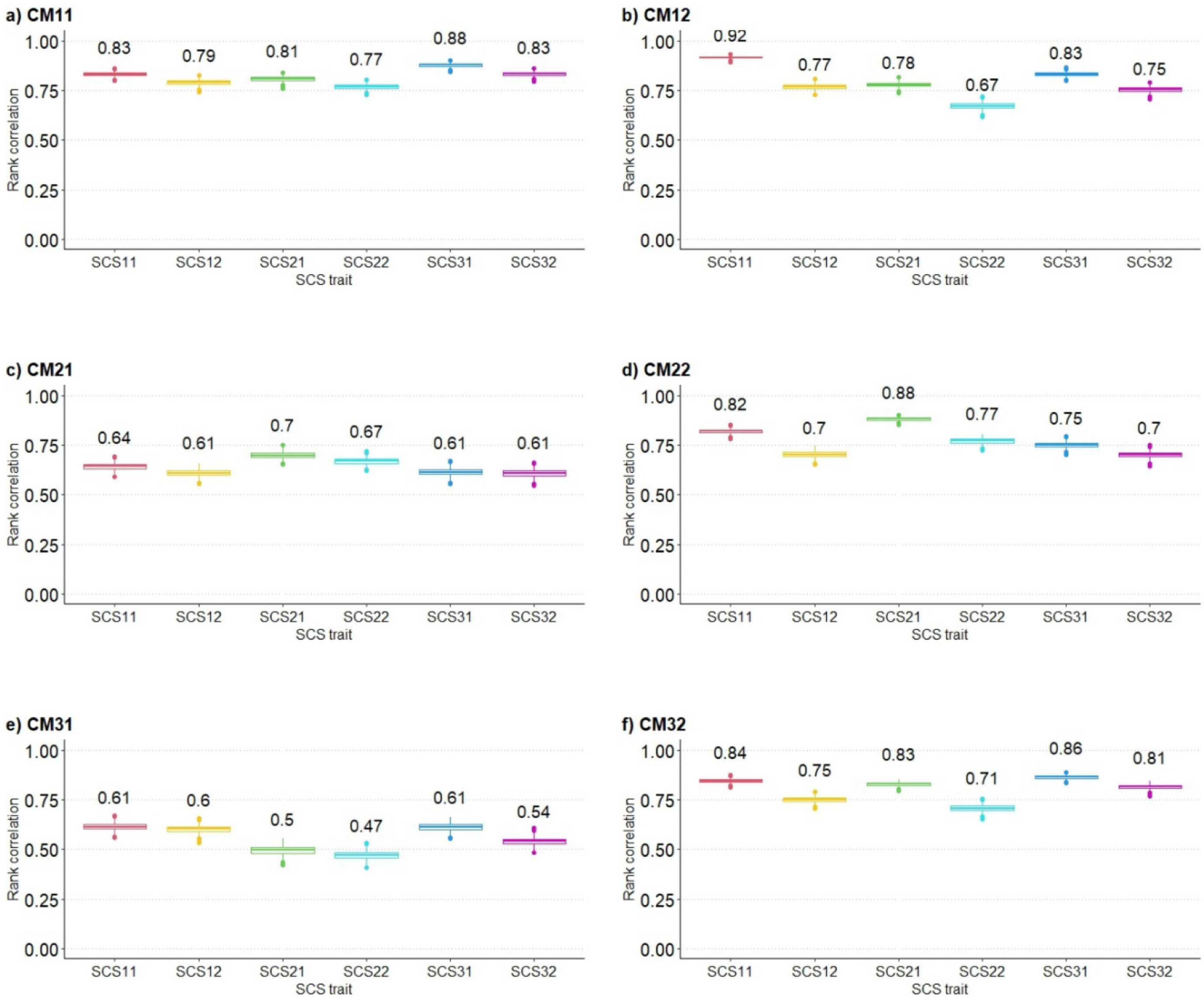

Figure 2. Box plot of rank correlation of sires for CM traits between univariate and bivariate analyses including SCS traits (each color denotes one particular SCS trait). See definition of traits in Table 1. In the box plots, the upper edge is the first quartile (Q1), the lower edge is the third quartile (Q3), the line inside the box is the median or second quartile (Q2), whiskers show the values within 1.5 times the interquartile range, from Q1 to Q3, and dots are outliers.

performed. For CM traits in early lactation (Figures 1a, 1c, and 1e), the improvement obtained from bivariate analyses with all SCS traits was very similar by number of daughters for CM11 (around 7%, 10%, and 12% more, respectively) and CM21 (around 16%, 23%,and 28% more, respectively). However, the advantages of the auxiliary SCS traits were remarkably high and different for CM31, even with 50 daughters or fewer, due to the low additive variance from the univariate analyses. Note that in Figure 1e there was an expected increase in reliability for SCS11 and SCS12 with more daughters, but it is not noticeable because of the scale. Increases of reliabilities for CM traits in late lactation (Figures 1b, 1d, and 1f), were quite stable regardless the lactation number for all SCS traits (13%–14%, 18%–19%, and 18%–20% more). There was an exception with SCS22 that could not improve the reliability of sires with fewer than 50 daughters obtained with the univariate analysis of CM32. Most available literature did not differentiate among lactations and milking periods as in our study, but other authors also found substantial increases for both cows and sires with the additional information of traits with higher heritability than CM. Zavadilová et al. (2020) reported mean reliabilities from the single-trait model of 16% for cows and 36% for sires, which increased up to 25% and 45%, respectively, when performing multitrait analyses.

Results published by Eding et al. (2009) showed even higher increases for bulls.

Comparing sires' ranking for CM using the EBV estimated with univariate analyses versus EBV from the bivariate analyses with SCS traits (Figure 2), Spearman's correlations ranged from 0.47 to 0.92. In general, rank correlations were lower in the first milking period than in the second period, in accordance with the genetic correlations and the reliabilities. In the first milking period, the higher correlations were for CM11, followed by CM21, and then CM31 (Figures 2a, 2c, and 2e). The highest rank correlations were obtained with the SCS trait that showed the lowest overall improvement of EBV reliability. In bivariate analysis both traits lend information to each other, and accuracy will increase with respect to univariate analysis depending on the trait with the highest heritability, and as much as the genetic correlation between them. However, the standard errors of the estimated genetic correlations were not considered, and this may explain that the SCS trait most correlated with CM a trait did not lead to the best rank correlation. We studied the rank correlation for the best 100 sires and the rank correlation for the worst 100 sires (Supplemental Figure S1; see Notes). It seemed that the discarding decision led to more similar results than when selecting candidates for sires of dams.

CONCLUSIONS

To our knowledge, there are no studies in the literature yet approaching the study of all CM traits combining early and late lactation and different lactations, following the harmonization suggested by the EuroGenomics Golden Standard. Regarding CM traits, CM in ≥ 3 lactations were not different from those in the second lactation. It has been shown that SCS traits can be helpful in mastitis resistance selection because the multitrait evaluation added substantial extra reliability of EBV for CM. However, SCS data from the same lactation and milking period as the CM diagnosis could not be the most informative, but SCS in the first 2 lactations and both milking periods seemed to be enough to predict all CM traits. We can conclude that SCS in first lactation could be useful to supplement CM data in the first and second lactations to improve udder health genetic evaluation. However, sire ranking is expected to vary because of the low heritability of CM, even with the additional SCS information.

NOTES

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Nonstandard abbreviations used: CM = clinical mastitis; CONAFE = Spanish Holstein Association; L = lactation; MKP = milking period; se = sensitivity; sp = specificity.

REFERENCES

Ali, A., and G. E. Shook. 1980. An optimum transformation for somatic cell concentration in milk. J. Dairy Sci. 63:487–490. https://doi.org/10.3168/jds.S0022-0302(80)82959-6.

Barkema, H. W., Y. H. Schukken, and R. N. Zadoks. 2006. *Invited review:* The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. J. Dairy Sci. 89:1877–1895. https://doi.org/10.3168/jds.S0022-0302(06)72256-1.

Bloemhof, S., G. De Jong, and Y. de Haas. 2009. Genetic parameters for clinical mastitis in the first three lactations of Dutch Holstein cattle. Vet. Microbiol. 134:165–171. https://doi.org/10.1016/j.vetmic.2008.09.024.

Bludau, M. J., A. Maeschli, F. Leiber, P. Klocke, J. A. Berezowski, M. Bodmer, and B. Vidondo. 2016. The influence of the rearing period on intramammary infections in Swiss dairy heifers: A cross-sectional study. Prev. Vet. Med. 129:23–34. https://doi.org/10.1016/j.prevetmed.2016.04.013.

Butler, D. G., B. R. Cullis, A. R. Gilmour, B. G. Gogel, and R. Thompson. 2023. ASReml-R Reference Manual Version 4.2. VSN International Ltd., Hemel Hempstead, HP2 4TP, UK. Accessed: Oct. 18, 2023. https://asreml.kb.vsni.co.uk/wp-content/uploads/sites/3/ASReml-R-Reference-Manual-4.2.pdf.

Carlén, E., E. Strandberg, and A. Roth. 2004. Genetic parameters for clinical mastitis, somatic cell score, and production in the first three lactations of Swedish Holstein cows. J. Dairy Sci. 87:3062–3070. https://doi.org/10.3168/jds.S0022-0302(04)73439-6.

Charfeddine, N., J. Blanco, and M. A. Peréz-Cabal. 2018. Genotyping and phenotyping of new health traits in Spanish dairy cattle herds. I-SA project. Page 197 in Book of Abstracts 69th EAAP Annual Meeting, Dubrovnik, Croatia.

Charfeddine, N., J. L. Tejeda, A. Gonzalez, and P. Codesido. 2013. Centralized electronic recording system of claw health data in Spain: I-SAP. Pages 101–103 in Proc. 9th International Conference on Lameness in Ruminants, Bristol, UK.

CONAFE. 2021. Recogida de información sobre Salud Animal I-SA. Definición de las patologías. Accessed Jul. 25, 2024. https://www.revistafrisona.com/DesktopModules/EasyDNNNews/DocumentDownload.ashx?portalid=0&moduleid=1468&articleid=4582&documentid=756.

CONAFE. 2023. Evaluación genética nacional del vacuno frisón español. Accessed Jul. 25, 2024. https://sinbad.conafe.com/IndicesGen/Docs/evaluaciones_Metodologia_y_requisitos.pdf.

de Haas, Y., W. Ouweltjes, J. ten Napel, J. J. Windig, and G. de Jong. 2008. Alternative somatic cell count traits as mastitis indicators for genetic selection. J. Dairy Sci. 91:2501–2511. https://doi.org/10.3168/jds.2007-0459.

Eding, H., Y. de Haas, and G. de Jong. 2009. Predicting mastitis resistance breeding values from somatic cell count indicator traits. Proceedings of the 2009 Interbull meeting N. 40. Accessed Jul. 22, 2024. https://journal.interbull.org/index.php/ib/article/view/1479.

Efron, B., and R. Tibshirani. 1986. Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. Stat. Sci. 1:54–75. https://doi.org/10.1214/ss/1177013815 https://www.jstor.org/stable/2245500.

Emanuelson, U., B. Danell, and J. Philipsson. 1988. Genetic parameters for clinical mastitis, somatic cell counts, and milk production esti-

mated by multiple-trait restricted maximum likelihood. J. Dairy Sci. 71:467–476. https://doi.org/10.3168/jds.S0022-0302(88)79576-4.
EuroGenomics. 2022a. Golden Standard for udder health. Accessed Dec. 2, 2022. https://www.eurogenomics.com/golden-standards.html.
EuroGenomics. 2022b. Memo on accounting for Heterogeneous Variance. Accessed Dec. 2, 2022. https://www.eurogenomics.com/golden-standards.html.
Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson. 2021. ASReml User Guide Release 4.2 Functional Specification, VSN International Ltd., Hemel Hempstead, HP2 4TP, UK. https://www.vsni.co.uk.
Harmon, R. J. 1994. Physiology of mastitis and factors affecting somatic cell counts. J. Dairy Sci. 77:2103–2112. https://doi.org/10.3168/jds.S0022-0302(94)77153-8.
Heikkilä, A.-M., E. Liski, S. Pyörälä, and S. Taponen. 2018. Pathogen-specific production losses in bovine mastitis. J. Dairy Sci. 101:9493–9504. https://doi.org/10.3168/jds.2018-14824.
Interbull. 2001. Routine genetic evaluation for udder health traits. May 2001. Accessed Feb. 6, 2024. https://interbull.org/static/mace_evaluations_archive/udder/utable1-012.html.
Interbull. 2009. Routine genetic evaluation for udder health traits. August 2009. Accessed Feb. 6, 2024. https://interbull.org/static/mace_evaluations_archive/udder/u-table1-093.html.
Interbull. 2023. National genetic evaluation forms provided by countries. Accessed Jul. 25, 2024. https://interbull.org/ib/geforms.
Koeck, A., B. Heringstad, C. Egger-Danner, C. Fuerst, P. Winter, and B. Fuerst-Waltl. 2010. Genetic analysis of clinical mastitis and somatic cells count traits in Austrian Fleckvieh cows. J. Dairy Sci. 93:5987–5995. https://doi.org/10.3168/jds.2010-3451.
López-Paredes, J. 2022. Estimación bayesiana de la prevalencia de mamitis subclínica en una población de vacuno de leche española. MS thesis. Complutense University of Madrid. Accessed Apr. 11, 2024. https://hdl.handle.net/20.500.14352/73934
Mrode, R. A., and G. J. T. Swanson. 1996. Genetic and statistical properties of somatic cell count and its suitability as an indirect means of reducing the incidence of mastitis in dairy cattle. Anim. Breed. Abstr. 64:847–857. Accessed Jul. 28, 2024. https://www.cabidigitallibrary.org/doi/full/10.5555/19980100162.
Negussie, E., I. Strandén, and E. A. Mäntysaari. 2008. Genetic analysis of liability to clinical mastitis, with somatic cell score and production traits using bivariate threshold-linear and linear-linear models. Livest. Sci. 117:52–59. https://doi.org/10.1016/j.livsci.2007.11.009.
Nielsen, C. 2009. Economic impact of mastitis in dairy cows. PhD Thesis. Department of Animal Breeding and Genetics. Swedish University of Agricultural Sciences, Uppsala, Sweden. Accessed Jul. 28, 2024. https://pub.epsilon.slu.se/1968/1/Christel_Nielsen_kappa.pdf.
Pérez-Cabal, M. A., I. Cervantes, J. P. Gutiérrez, J. López-Paredes, and N. Charfeddine. 2023. Approaching the genetic evaluation models for clinical mastitis in Spanish dairy cows. Poster 23 in Book of Abstracts of EAAP 74th Annual Meeting 2023, Lyon, France.
Pérez-Cabal, M. A., G. de los Campos, A. I. Vazquez, D. Gianola, G. J. M. Rosa, K. A. Weigel, and R. Alenda. 2009. Genetic evaluation of susceptibility to clinical mastitis in Spanish Holstein cows. J. Dairy Sci. 92:3472–3480. https://doi.org/10.3168/jds.2008-1978.
Polat, B., A. Colak, M. Cengiz, L. E. Yanmaz, H. Oral, A. Bastan, S. Kaya, and A. Hayirli. 2010. Sensitivity and specificity of infrared thermography in detection of subclinical mastitis in dairy cows. J. Dairy Sci. 93:3525–3532. https://doi.org/10.3168/jds.2009-2807.
R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Sadeghi, H., U. Yáñez, A. I. De Prado, F. Gharagozlou, J. J. Becerra, P. G. Herradón, A. I. Peña, and L. A. Quintela. 2021. Effect of subclinical mastitis on reproductive performance of Holstein dairy cows in the Northwest of Spain. Span. J. Agric. Res. 19:e04SC01. https://doi.org/10.5424/sjar/2021194-18058.
Schepers, A. J., T. J. G. M. Lam, Y. H. Schukken, J. B. M. Wilmink, and W. J. A. Hanekamp. 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. J. Dairy Sci. 80:1833–1840. https://doi.org/10.3168/jds.S0022-0302(97)76118-6.
Schukken, Y. H., J. Hertl, D. Bar, G. J. Bennett, R. N. González, B. J. Rauch, C. Santisteban, H. F. Schulte, L. Tauer, F. L. Welcome, and Y. T. Gröhn. 2009. Effects of repeated gram-positive and gram-negative clinical mastitis episodes on milk yield loss in Holstein dairy cows. J. Dairy Sci. 92:3091–3105. https://doi.org/10.3168/jds.2008-1557.
Schutz, M. M. 1994. Genetic evaluation of somatic cell scores for United States dairy cattle. J. Dairy Sci. 77:2113–2129. https://doi.org/10.3168/jds.S0022-0302(94)77154-X.
Schwarz, D., U. S. Diesterbeck, S. Konig, K. Brugemann, K. Schlez, M. Zschock, W. Wolter, and C. P. Czerny. 2011. Microscopic differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. J. Dairy Res. 78:448–455. https://doi.org/10.1017/S0022029911000574.
Sears, P. M., and L. E. Heider. 1981. Detection of mastitis. Vet. Clin. North Am. Large Anim. Pract. 3:327–346. https://doi.org/10.1016/S0196-9846(17)30133-7.
Sears, P. M., D. J. Wilson, R. N. Gonzalez, and D. D. Hancock. 1991. Microbiological results from milk samples obtained premilking and post milking for the diagnosis of bovine intramammary infections. J. Dairy Sci. 74:4183–4188. https://doi.org/10.3168/jds.S0022-0302(91)78613-X.
Sharma, N., N. K. Singh, and M. S. Bhadwal. 2011. Relationship of somatic cell count and mastitis: An overview. Asian-Australas. J. Anim. Sci. 24:429–438. https://doi.org/10.5713/ajas.2011.10233.
Singh, A. K. 2022. A comprehensive review on subclinical mastitis in dairy animals: Pathogenesis, factors associated, prevalence, economic losses and management strategies. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 17:17057. https://doi.org/10.1079/cabireviews202217057.
Vazquez, A. I., D. Bates, S. Avadhanam, P. Perez Rodriguez, and G. Gorjanc. 2023. Package ‘pedigreeTools’. Accessed Feb. 6, 2024. https://doi.org/10.32614/CRAN.package.pedigreeTools.
Vazquez, A. I., K. A. Weigel, D. Gianola, D. Bates, M. A. Pérez-Cabal, G. Rosa, and Y. M. Chang. 2009. Poisson versus threshold models for genetic analysis of clinical mastitis in US Holsteins. J. Dairy Sci. 92:5239–5247. https://doi.org/10.3168/jds.2009-2085.
Zavadilová, L., E. Kašná, Z. Krupová, and M. Brzáková. 2020. Genetic parameters for clinical mastitis in Czech Holstein cattle. Czech J. Anim. Sci. 65:463–472. https://doi.org/10.17221/151/2020-CJAS.
Zavadilová, L., E. Kašná, Z. Krupová, and A. Klímová. 2021. Health traits in current dairy cattle breeding: A review. Czech J. Anim. Sci. 66:235–250. https://doi.org/10.17221/163/2020-CJAS.
Zavadilová, L., M. Stipkova, A. Svitakova, Z. Krupova, and E. Kasna. 2017. Genetic parameters for clinical mastitis, fertility and somatic cell score in Czech Holstein cattle. Ann. Anim. Sci. 17:1007–1018. https://doi.org/10.1515/aoas-2017-0006.
Zecconi, A., G. Sesana, D. Vairani, M. Cipolla, N. Rizzi, and L. Zanini. 2019. Somatic cell count as a decision tool for selective dry cow therapy in Italy. Ital. J. Anim. Sci. 18:435–440. https://doi.org/10.1080/1828051X.2018.1532328.
Zwald, N. R., K. A. Weigel, Y. M. Chang, R. D. Welper, and J. S. Clay. 2006. Genetic analysis of clinical mastitis data from on-farm management software using threshold models. J. Dairy Sci. 89:330–336. https://doi.org/10.3168/jds.S0022-0302(06)72098-7.

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