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Usability of bacteriological milk analyses for genetic improvement of udder health in Austrian Fleckvieh cows

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ABSTRACT

In addition to somatic cell count records and clinical mastitis diagnoses, results of bacteriological milk analyses provide valuable information regarding udder health. The pathogen causing an udder infection is currently not considered in Austria as part of the information used for estimation of routine breeding values for mastitis resistance. Therefore the objective of this study was to estimate heritabilities for, and genetic correlations between, udder traits of bacterial infection (bacterial infection, gram-positive and gram-negative bacterial infection) and routinely recorded udder health traits [acute mastitis, chronic mastitis, culling due to udder health problems, and somatic cell score (SCS)] in Austrian Fleckvieh cows. The basis for the genetic analyses was a data set with results from bacteriological milk analyses collected from 237 dairy farms and 6,822 cows over a period of 1 yr. Traits were defined as binary, apart from SCS, for which measures were available continuously. Multivariate analyses using a linear animal model were applied for estimating genetic parameters. The heritabilities for the occurrence of bacterial udder infection traits were 0.01. Heritabilities were 0.04 for acute mastitis, 0.02 for chronic mastitis, 0.02 for culling due to udder health problems, and 0.20 for SCS. Genetic correlations between bacteriological infection and the routinely recorded udder health traits were positive and ranged from 0.62 to 0.96. The genetic correlation between gram-positive and gram-negative bacterial infection was −0.20. The genetic correlation between acute and chronic mastitis was also close to zero. These results show that mastitis caused by different pathogens may be seen as different traits. As analyses were based on a relatively small data set and results were associated with rather high standard errors, further research with a larger data set should be carried out to confirm these results.

Key words: pathogen-specific mastitis, culture milk sample, genetic analysis, dairy cattle

INTRODUCTION

Mastitis is one of most frequent diseases in dairy cows worldwide. In Austrian dairy herds, udder health problems are a main reason for culling. In 2019, 14% of culled Fleckvieh cows were removed from the herd because of udder disease (ZuchtData, 2019).

In Austrian dairy cattle, breeding selection for improved udder health is based on direct and indirect information. In Austria a health monitoring system for cattle started in 2006 in which diagnoses from veterinarians are recorded (Egger-Danner et al., 2010, 2012). A standardized diagnosis key is used, consisting of 10 disease groups covering 65 different disease codes. The diagnosis data from veterinarians are collected monthly by the milk recording technicians or are sent electronically to the database by the veterinarians. Since 2011 recording of direct health traits is integrated into the breeding programs of Austrian cattle breeders and therefore compulsory for all breeding herds. For the routine genetic evaluation, clinical mastitis is defined as a binary trait. Cows that have at least one treatment of clinical mastitis (acute or chronic) by a veterinarian or that are culled because of udder health problems in the observation period −10 to 150 d after calving are treated as diseased. With a value of 0.02, the heritability of clinical mastitis based on a linear animal model is low (Fuerst et al., 2011). Clinical mastitis breeding values as well as breeding values for SCS, fore udder attachment, udder depth, and front teat length are combined into an udder health index (Fuerst and Egger-Danner, 2014). Since April 2016, the udder health index has an economic weighting of 10% in the total merit index in Austrian Fleckvieh (Fuerst-Waltl et al., 2016). Finding new phenotypes to select for im-

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proved mastitis resistance remains an important issue. Potential has been seen, for example, in the use of data derived from bacteriological milk analyses (Martin et al., 2018). Several genetic analyses for the most common pathogens (*Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Escherichia coli*, coagulase-negative staphylococci), as well as for various groups of pathogens (environmental or contagious, gram-negative or gram-positive bacteria) associated with clinical or subclinical mastitis (e.g., Sørensen et al., 2009a,b; Haugaard et al., 2012, 2013) have been published, assuming that differences in cows' pathogen defenses are due to genetic variations. These studies from different countries and on different breeds have revealed low heritabilities but differences in genetic variance depending on the pathogen (group) involved. So far, information on the pathogen responsible for an udder infection has not been included in any breeding program (Martin et al., 2018). Challenges include the difficulty of and high costs for comprehensive data collection (Rupp and Boichard, 2003; Detilleux, 2009).

Bacteriological milk analysis is used as an important diagnostic tool for the identification of causative mastitis pathogens (Griffioen et al., 2018). The knowledge of the mastitis-causing pathogen is relevant in the context of encouraging a more prudent use of antimicrobials (Krömker and Leimbach, 2017). Although the use of veterinary antimicrobials in food-producing animals is relatively low in Austria compared with other European countries (EMA, 2019), Firth et al. (2017) showed that around one-third of all antimicrobial doses administered within a study population of 248 farms were used to treat udder disease in lactating cows.

Bacteriological milk analysis is voluntary in Austria. Several laboratories offer bacteriological milk analyses to farmers. Within the project ADDA—Advancement of Dairying in Austria, standardized diagnostic codes for results of bacteriological milk analyses were developed to ensure harmonized data with respect to animal-specific information and nomenclature of pathogens. Interfaces were implemented to integrate laboratory data within the national cattle database (Obritzhauser et al., 2017), and a standardized data set was subsequently available in Austria for the first time.

The objective of this study was to analyze the usability of bacteriological milk analyses as an additional direct measure for genetic improvement of udder health in Austrian Fleckvieh cattle. In a first step, investigations were carried out on how to define suitable pathogen-specific traits. In a second step, heritabilities for, and genetic correlations between, pathogen-specific traits and clinical mastitis, culling due to udder health problems, and SCS, were estimated.

MATERIALS AND METHODS

In accordance with national legislation and good scientific practice guidelines, the study was registered with the Institutional Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna (ref no. ETK-13/11/2015). No invasive procedures were performed as part of this study.

Data

A comprehensive data set on udder health data from 248 farms, including results of bacteriological milk analyses, was available for our study. Active documentation of health data based on veterinary treatments was an essential prerequisite for the participation in the project, to ensure high data quality. Intensive training and support activities took place as part of the project, to ensure consistent data recording. Farmers and veterinarians joining the project were requested to provide milk samples from cows with clinical mastitis or whenever udder health problems (e.g., increased SCC, visible change in the udder or milk) were suspected. In most cases milk was taken from all quarters, even from healthy ones. The milk samples were analyzed at milk laboratories throughout the country according to standardized methods (Obritzhauser et al., 2017). Results were submitted to the central cattle database. Each data set contained the identification number of farm, cow, and laboratory, date of sampling, and pathogen-specific code, as well as pathogen denotation at quarter level and quarter location. A total of 6,892 quarter milk samples from 1,382 cows and 248 dairy farms were collected over a 1-yr period between October 1, 2015, and September 30, 2016. In all, 450 samples were discarded because of mixed or contaminated flora (5.1%), or empty or shattered vials or poor milk quality (1.4%) that prevented laboratory analysis. The majority (71.2%) of the analyzed quarter milk samples were culture-negative. A total of 1,533 milk samples (22.3%) were culture-positive, including isolates from more than 20 different mastitis pathogens. The most frequently isolated genus was *Staphylococcus* (54.0%), of which CNS (466 quarters) and *Staph. aureus* (338 quarters) were the most common species. Within *Streptococcus* spp. (25.2%) *Strep. uberis* (108 quarters), *Strep. dysgalactiae* (77 quarters), and other streptococci (201 quarters) were most common. In enterobacteria (13.4%), *E. coli* (99 quarters) was the species most commonly found. An overview of the most frequently isolated bacteria is given in Table 1. For further analyses, bacteriological milk analyses were considered at cow level. The number of infected quarters and quarter location were ignored.

Table 1. Culture results from quarter milk samples submitted to diagnostic laboratories from October 2015 to September 2016 in 248 Austrian dairy farms (quarter level): results from initial (not edited) data set

Item	Positive samples (n = 1,533)	All samples (n = 6,892)
Gram-positive bacteria, %		
Coagulase-negative staphylococci	30.4	7.2
Staphylococcus aureus	22.1	5.2
Streptococcus spp. (other than *dysgalactiae* or *uberis*)	13.1	3.1
Streptococcus uberis	7.0	1.7
Streptococcus dysgalactiae	5.0	1.2
Corynebacterium spp.	3.7	0.9
Enterococcus spp.	2.4	0.6
Gram-negative bacteria, %		
Enterobacter spp.	7.1	1.7
Escherichia coli	6.3	1.5
Others,[1] %	2.9	0.7

[1]Includes gram-positive bacteria and nonbacterial pathogens (e.g., yeast, prototheca).

In addition to animal information (e.g., herd, calving date, parity, breed, pedigree) routinely recorded udder health information (Egger-Danner et al., 2012) such as veterinary diagnoses of clinical mastitis (acute and chronic mastitis), reasons for culling, and SCC from milk test-day records were provided by ZuchtData (Vienna, Austria). Test-day SCC recording followed ICAR AT4, AT5, or AT6 in an a.m./p.m. recording scheme (www.icar.org).

Trait Definition

Pathogen-Specific Mastitis Traits. Although some pathogens were detected frequently, their occurrence was too low to estimate genetic parameters for a single pathogen. For this reason, 3 traits for susceptibility to groups of pathogens have been defined: (1) gram-positive bacteria (**GRAM+**), (2) gram-negative bacteria (**GRAM−**; see Table 1), and (3) any bacterial infection (**BACI**). The trait GRAM+ includes records on staphylococci, streptococci, corynebacteria, and enterococci, and the trait GRAM− records on enterobacteria. The trait BACI includes all culture-positive milk samples. The pathogen-specific mastitis traits were defined as binary, 1 or 0, depending on whether or not the cow had at least one culture-positive quarter with the defined pathogen-specific group at least once during lactation (−10 to 305 DIM). Cows were required to be at least 1 d in milk within the period of risk; otherwise the trait was set to missing. More rigorous data preparation was not possible; otherwise much data would be lost due to the small number of farms and cows.

Routinely Recorded Udder Health Traits. Additional investigations were carried out for the traits acute mastitis (**AcM**) and chronic mastitis (**ChM**). In Austria, dispensing of antimicrobial treatments is strictly regulated by law. These treatments are available only from veterinarians and only after clinical diagnosis. If farmers notice signs of inflammation in the udder, they contact their veterinarian for a farm visit. Acute mastitis and chronic mastitis are recorded in the Austrian health monitoring system with separate codes entered by the respective veterinarian. The distinction between acute and chronic mastitis is based on veterinary diagnosis and the lactation history of the animals. The study was observational, and no predefined criteria were set with respect to veterinary diagnoses. Each veterinarian, therefore, diagnosed mastitis according to his or her veterinary experience and clinical examination of the animal. Culling due to udder problems (**CULL**) was based on farmer information. The farmer may give only one culling reason for each cow at the time of removal. For all described traits lactation records from −10 to 305 DIM were considered, scored as 1 in case of disease or culling and 0 otherwise. Only cows that had completed at least two-thirds of the opportunity period (200 d) on the farm were considered healthy. This should ensure that each cow has a sufficient chance to express the trait.

For analyses, SCC records were transformed by the formula $SCS = \log_2 (SCC/100{,}000) + 3$ to achieve an approximately normal distribution. A lactation mean SCS (**LSCS**) considering all test-day SCS from 5 to 305 d after calving was defined. At least 5 SCS records had to be available; otherwise this trait was set to missing.

Data Editing. Bacteriological milk analyses from 248 farms were collected from October 1, 2015, and September 30, 2016, whereas for the routinely recorded udder health traits a longer observation period was considered (cows calving between January 1, 2013, and March 31, 2017) to ensure more reliable estimates for these traits. For the analysis, only farms actively participating in the health monitoring system and collect-

ing health data were considered. For farms that had at least one clinical mastitis diagnosis between October 1, 2015, and September 30, 2016, but without any bacteriological milk analysis, the traits GRAM+, GRAM−, and BACI were set to missing, to reduce false-negative cases. Analyses were carried out for Fleckvieh cows, from all parities, with a maximum foreign gene proportion of <50%. Records from −10 to 305 DIM were considered. Age at first calving was limited to 18 to 47 mo, and only cows with a calving interval shorter than 800 d were included. Cows were excluded if pedigree information was missing. After edits, a total of 16,251 records from 6,822 Fleckvieh cows from 237 herds were used for analyses. Not every cow had records available for every trait. A summary of the analyzed data sets is given in Table 2. Animal pedigree files were generated by tracing back 5 generations. The number of animals included in total pedigree of the animal model was 28,733.

Model

Genetic Analyses. Data were analyzed with multivariate linear animal models using the software package VCE-6 (version 6.0.2; Groeneveld et al., 2008). As the assumption of normally distributed data is not fulfilled, threshold models are, at least in theory, more appropriate to analyze binary traits. In the present study, linear models were applied, because numerous authors have found that linear models are robust toward departures from normality and performed equally well as threshold models (e.g., Negussie et al., 2008).

Multivariate analyses were carried out for the trait combinations as follows: (1) BACI, AcM, ChM, CULL, and LSCS; (2) GRAM+, GRAM−, AcM, ChM, CULL, and LSCS. The following model was applied to all traits:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_h h} + \mathbf{Z_{pe} pe} + \mathbf{Z_a a} + \mathbf{e},$$

where y is the observation of interest; $\boldsymbol{\beta}$ is a vector of systematic effects, including fixed effects of foreign gene proportion, year-month of calving, parity-age at calving, laboratory-year (only for GRAM+, GRAM−, and BACI), and type of recording-year (only for AcM and ChM); **h** is a vector of random herd-year effects; **pe** is a vector of random permanent environmental effects; **a** is a vector of random animal effects; **e** is a vector of random residuals; and $\mathbf{X}$, $\mathbf{Z_h}$, $\mathbf{Z_{pe}}$, and $\mathbf{Z_a}$ are the corresponding incidence matrices.

Foreign gene proportion was grouped into 3 classes: 1 = 0%, 2 = 1 to 25%, and 3 = 26 to <50%. Six calving-age classes were formed for each of the first 2 parities. Parity-age was classified into <26, 27 to 28, 29 to 30, 31 to 32, 33 to 34, and >34 mo for first-calving cows; and age at second calving was grouped into <39, 40 to 41, 42 to 43, 44 to 45, 46 to 47, and >47 mo. For older cows, parity-age classes were 3, 4, and 5+. Type of recording was defined in 3 classes: 1 = less than 25% of the diagnoses of a farm are electronically transmitted, 2 = 25 to 75% of the diagnoses of a farm are electronically transmitted, and 3 = more than 75% of the diagnoses of a farm are electronically transmitted.

Heritability Transformation. Estimated linear heritabilities for the incidence of bacterial infection, acute mastitis, chronic mastitis, and culling due to udder health problems were transformed by the following formula of Dempster and Lerner (1950) to gain comparable estimates on the underlying scale:

$$h_{th}^2 = h_l^2 \left[q(1-q)\right] / z^2,$$

where h_{th}^2 is the heritability in threshold model and h_l^2 the estimated linear heritability for binary udder health

Table 2. Numbers of records, cows, sires, and herds of data sets with different length of observation period for genetic analyses of different udder health traits in Austrian Fleckvieh cows

Trait	Mean	Records	Cows	Sires	Herds
BACI,[1] %	7.2	6,900	4,605	721	182
Gram+,[1] %	6.2	6,900	4,605	721	182
Gram−,[1] %	1.3	6,900	4,605	721	182
AcM,[2] %	13.0	13,473	5,989	854	232
ChM,[2] %	2.4	13,193	5,926	848	231
CULL,[2] %	2.9	13,438	5,978	853	231
LSCS[3]	2.1	10,393	5,984	833	237
Total		16,251	6,822	860	237

[1]Cows with at least one record on gram-positive (Gram+), gram-negative (Gram−), or any bacterial infection (BACI) between October 1, 2015, and September 30, 2016. Defined as binary (0 or 1) traits.

[2]Cows with at least one record on acute (AcM) or chronic (ChM) mastitis, or culling due to udder health problems (CULL), calving between January 1, 2013, and March 31, 2017. Defined as binary (0 or 1) traits.

[3]Lactation mean SCS (LSCS) from 5 to 305 d after calving, recorded at test-days between January 1, 2015, and March 31, 2017.

traits, q is the frequency of infection or disease, and z^2 represents the ordinate at standard normal distribution corresponding to the probability of occurrence of q. Standard error of heritability was transformed or calculated in the same way:

$$SE_{th}^2 = SE_l^2 \left[q(1-q)\right] / z^2 ,$$

where SE_{th} is the standard error in threshold and SE_l the standard error in linear model.

RESULTS AND DISCUSSION

Phenotypic Description

In the current study, milk samples were collected for bacteriological culture in case of clinical (acute or chronic) mastitis or suspected udder health problems (e.g., increased SCC). *Staphylococcus aureus*, *Strep. uberis*, CNS, *Strep. dysgalactiae*, *E. coli*, and other streptococci had the highest incidence (Table 1). Similar to our study, *Staph. aureus* and CNS were the most prevalent mastitis pathogens identified in studies from Germany (Schafberg et al., 2006), Norway (Østerås et al., 2006; Haugaard et al., 2012; Haugaard et al., 2013), and Sweden (Holmberg et al., 2012). Different streptococci species and *E. coli* were most often isolated from culture samples in a study from US dairy herds (Cha et al., 2016). *Streptococcus uberis* and *E. coli* dominated in Belgian Holstein cows (Verbeke et al., 2014), *Strep. uberis* and *Staph. aureus* in Danish Holstein cows (Sørensen et al. 2009a), and *Staph. aureus* and *E. coli* in a study from the Netherlands (de Haas et al., 2003). For subclinical mastitis, staphylococci species predominated (Holmberg et al., 2012; Haugaard et al., 2013), whereas clinical mastitis was more often associated with higher frequencies of streptococci species and *E. coli* (Sørensen et al., 2009a; Haugaard et al., 2012; Verbeke et al., 2014).

A large proportion of the milk samples were collected at the beginning as well as toward the end of the lactation period (see Figure 1). The reason for this is that mastitis occurs more frequently at the beginning of lactation. At the end of lactation, cows are sampled more frequently for control before drying off. The proportion of culture-positive results was highest (>60%) in early lactation, which is in common with the occurrence of clinical mastitis diagnoses (see Table 3) found in this study. Of 1,382 cows sampled, 846 (about 55%) had at least one culture-positive quarter sample. Hindquarters were found to be infected more often (58%) than forequarters (42%).

The overall frequency of bacterial infection was 7.2% (Table 2). Infections with gram-positive pathogens,

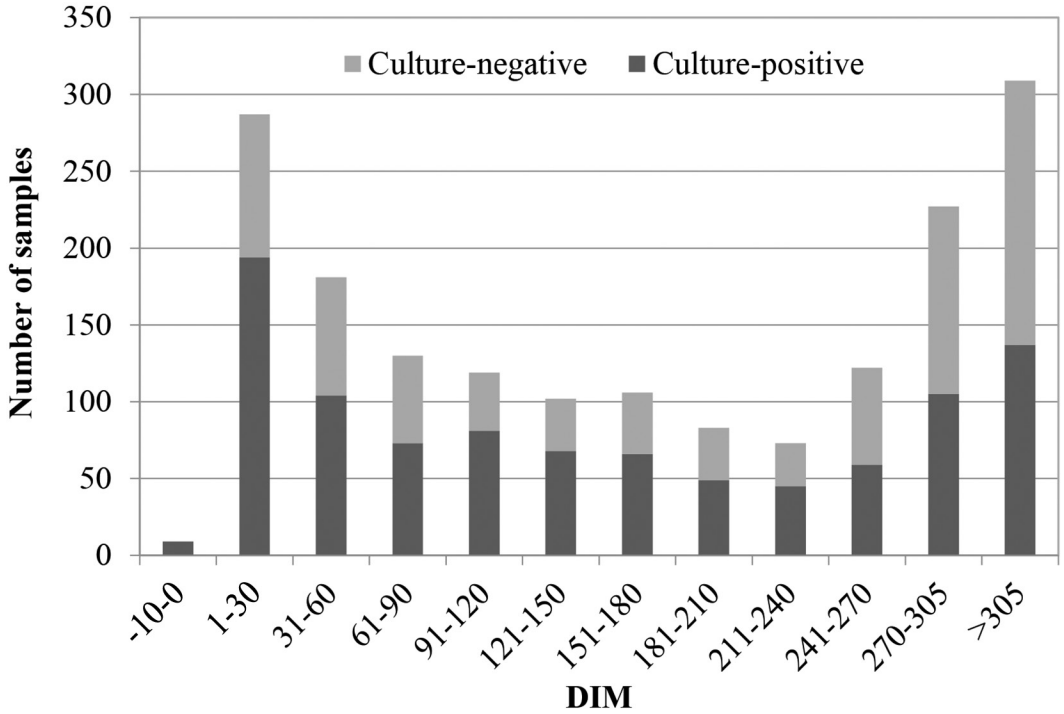

Figure 1. Number of milk samples analyzed (cow level) during the 1-yr collection period of all cows (no restriction with respect to breed) and parities by DIM after calving.

which can lead to acute and chronic mastitis, were far more frequent (6.2%) than with gram-negative ones (1.3%), which predominantly lead to acute mastitis (Schukken et al., 1997).

The frequencies of AcM, ChM, and CULL were 13.0, 2.4, and 2.9%, respectively (Table 2). Higher clinical mastitis frequencies were recorded in British Holstein cows (first to third lactation, 14.0–25.9% within 0–305 DIM; Pritchard et al., 2013) and in German Holstein cows (38.5%, at least one treatment within 1–305 DIM; Martin et al., 2013).

The mean score for LSCS was 2.05. The average SCC of Fleckvieh cows in Austria is 183,332 cells/mL (ZuchtData, 2019), which corresponds to an SCS of 3.8.

As shown in Table 3, the majority of bacteriological infections occurred in early lactation. Another peak of infections could be observed toward the end of lactation, particularly for infections caused by gram-positive bacteria. In a large dairy herd in Germany, staphylococci infections frequently occurred in an early lactation stage, with a decreasing trend over the course of the lactation period (Schafberg et al., 2006). A similar pattern was observed in Norwegian dairy cattle (Østerås et al., 2006). The opposite was observed for *Strep. uberis* and *Strep. dysgalactiae*, whereas CNS was commonly found toward the end of lactation (Østerås et al., 2006).

Figure 2 shows that cows with a culture-positive milk sample had on average a higher SCS throughout the lactation period than cows with a culture-negative milk sample or those where no bacteriological milk analysis was carried out. Cows with a culture-negative sample also had a higher mean SCS, in contrast to cows without a culture sample. This could be due to cases of false-negative results (pathogen is present but has not been detected), or the milk sample may have been collected after a mastitis treatment, where pathogens were no longer present but the SCC had not yet returned to its normal state.

As shown in Figure 3, differences in the course of average SCS during lactation between infections with gram-positive and gram-negative bacteria were marginal. As expected, a high mean SCS throughout the lactation period was observed for cows with at least one record on acute or chronic mastitis and of cows culled due to udder health problems. Cows with chronic mastitis and those infected with gram-negative bacteria showed the highest mean SCS. De Haas et al. (2004) analyzed associations between different SCC patterns based on test-day records and occurrence of clinical mastitis due to the most prevalent pathogens. Typical SCC characteristics were found for *Staph. aureus* (prolonged but moderate increase) and *E. coli* (high but brief increase). Streptococci species could not be clearly assigned to any SCC patterns (de Haas et al., 2004). Nash et al. (2000) reported a higher average SCS (total lactation) in Holstein cows with an udder infection caused by major pathogens (streptococci, coliforms) than in cows with an infection caused by minor pathogens (CNS) in an early lactation stage.

Genetic Parameters

Heritabilities for Susceptibility to Pathogen-Specific Mastitis Traits. Estimates of heritabilities for analyzed traits are given in Table 4. Heritabilities for BACI, GRAM+ and GRAM− were 0.01. Schafberg et al. (2006) defined a trait for the occurrence of intramammary infections using foremilk samples from 786 dairy cows from a large single farm in Germany and determined a heritability estimate of 0.09. This value is significantly higher than the corresponding value of the present study.

The heritability estimate for BACI was 0.04 when transformed from the observable to the underlying scale (see Table 4), and thus was similar to the threshold model estimates for bacterial infection (0.04–0.06)

Table 3. First identification (%) of infection with gram-positive bacteria (Gram+), infection with gram-negative (Gram−) bacteria, any bacteriological infection, acute and chronic mastitis diagnoses, and culling due to udder health problems by stage of lactation, measured as days after calving

Days postpartum	Gram+ infection (n = 431)	Gram− infection (n = 92)	Bacteriological infection (n = 501)	Acute mastitis (n = 1,778)	Chronic mastitis (n = 325)	Culling (n = 1,433)
−10–0	3.2	0.0	2.8	4.9	2.2	0.4
1–30	23.9	34.8	23.8	32.6	23.4	11.7
31–60	12.5	13.0	12.0	13.2	19.7	8.7
61–90	8.6	8.7	8.8	9.8	8.6	8.7
91–120	8.4	5.4	8.6	8.3	9.5	6.4
121–150	7.4	9.8	7.8	7.1	8.3	8.7
151–180	6.3	5.4	7.4	6.3	4.6	10.3
181–210	6.7	9.8	6.6	5.4	6.8	10.5
211–240	4.2	9.8	4.2	4.0	5.2	11.4
241–270	6.5	5.4	6.8	4.2	3.4	10.0
270–305	12.3	3.3	11.4	4.0	8.3	13.2

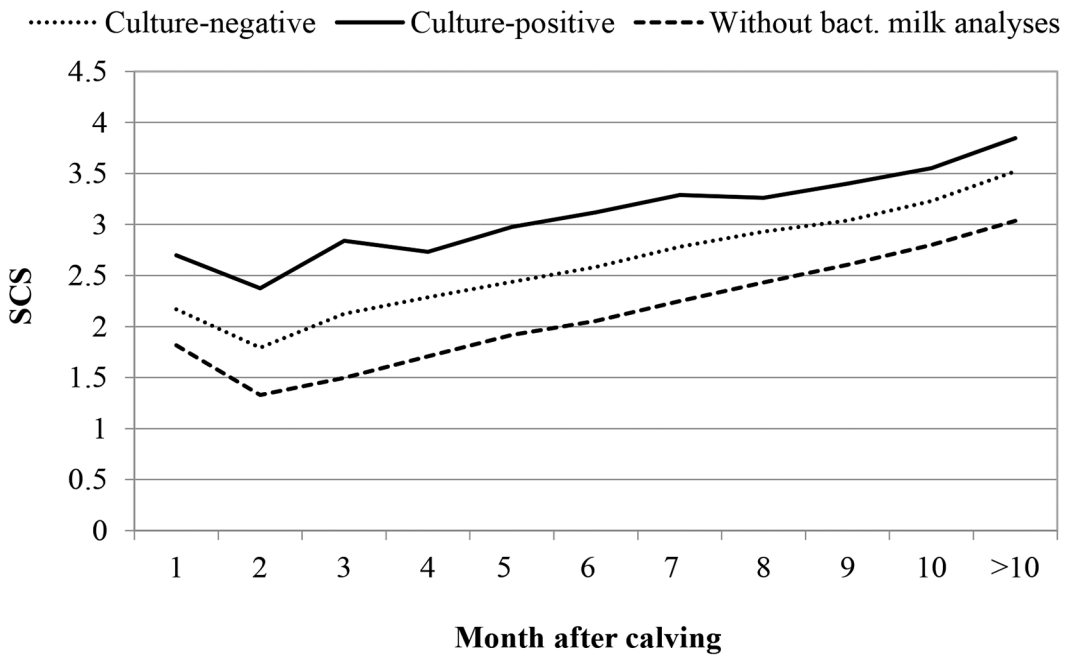

Figure 2. Mean SCS of Austrian Fleckvieh cows with positive, negative, or without bacterial (bact.) culture sample during lactation.

determined in other studies (Schafberg et al., 2006; Sørensen et al., 2009a).

In our study, the transformed threshold heritability estimates for GRAM+ and GRAM− were 0.05 and 0.13, respectively (Table 4). Studies from Denmark, the Netherlands, and Norway investigated several pathogen-specific clinical and subclinical mastitis traits (*Staph. aureus*, *Strep. uberis*, *Strep. dysgalactiae*, CNS, and *E. coli*.), as well as further pathogen group-specific clinical mastitis traits (contagious, environmental), and found heritability estimates in the range of 0.02 and 0.14 (de Haas et al., 2002; Sørensen et al. 2009a; Haugaard et al., 2012, 2013) using logit or probit threshold sire models. Overall, heritabilities of the traits based on results of bacteriological milk analyses were low but showed that genetic variation exists for selection.

Heritabilities for Other Routinely Recorded Udder Health Traits. Heritabilities for AcM, ChM, and CULL were slightly higher than those for susceptibility to pathogen-specific mastitis traits, with 0.04, 0.03, and 0.02, respectively (see Table 4). As expected, the highest heritability of 0.20 was estimated for LSCS. Heritabilities for clinical mastitis traits (AcM and ChM) and LSCS were in the same range compared with previous Austrian studies on more comprehensive data sets (Koeck et al., 2010; Fuerst et al., 2011; Pfeiffer et al., 2015). The heritability estimates for mastitis were also in agreement with results reported from Canadian and Spanish Holstein cows (h^2 = 0.04; Pérez-Cabal and Charfeddine, 2013; Pritchard et al., 2013). Heritability for mean LSCS was higher than previously reported values for mean lactation SCS, ranging from 0.12 to 0.17 (Negussie et al., 2008; Martin et al., 2013; Pritchard et al., 2013). By contrast, Bloemhof et al. (2009) determined a value of 0.35 for mean SCS from 5 to 335 d after calving in Dutch Holstein cows using a linear sire model.

Genetic Correlations. Genetic correlations between traits are given in Tables 5 and 6. Although most estimates were significantly different from 0, it should be noted that frequencies were low for some traits, especially for GRAM−. When interpreting the results, the relatively low number of GRAM− samples should be kept in mind. Slightly negative genetic correlations were estimated between GRAM+ and GRAM− (−0.20), indicating differences in genetic control. This is in common with results of immunological and microbiological studies (Bannerman et al., 2004; Griesbeck-Zilch et al., 2008; Bhattarai et al., 2018) but in contrast to the genetic analysis of Sørensen et al. (2009a). They reported an unexpectedly high genetic correlation of 0.73 between gram-positive and gram-negative pathogens; however, the authors exclusively referred to clinical mastitis, which limits the comparability of these values with the current study. Gram-negative bacteria often cause acute infections and specifically affect mechanisms of the innate defense system. In contrast, gram-positive bacteria more often lead to chronic infections and cause reactions of the specific immune system (Schukken et al., 1997).

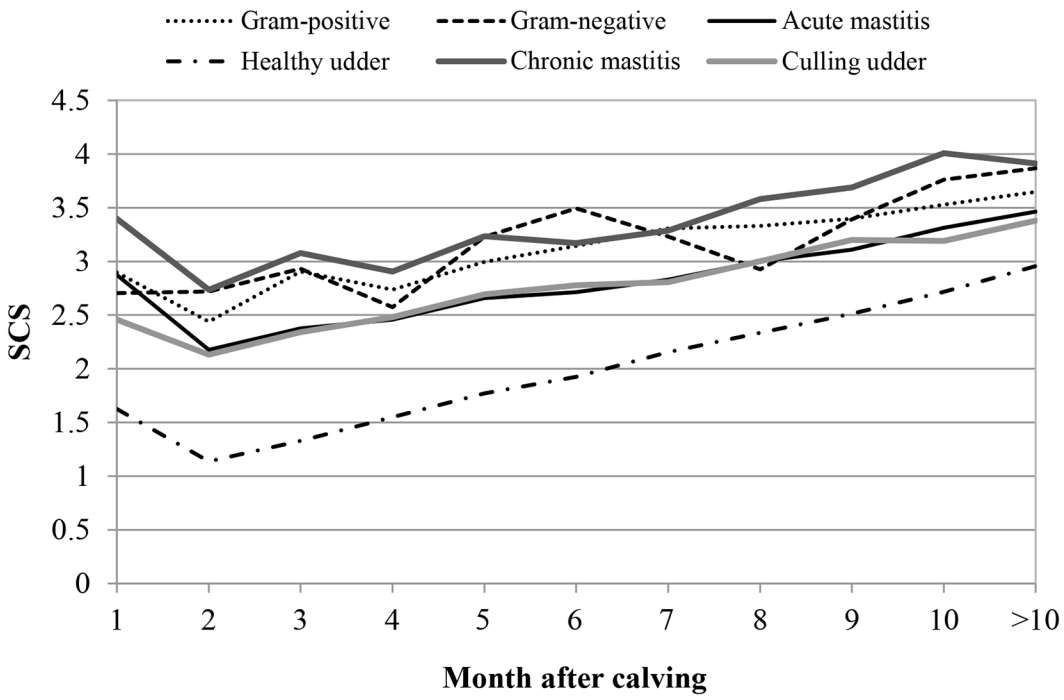

Figure 3. Mean SCS of Austrian Fleckvieh cows with gram-positive or gram-negative culture samples, acute or chronic mastitis, culling due to udder health problems, or a healthy udder (without infection, mastitis diagnosis, or culling due to udder problems) during lactation.

Positive and medium-to-high genetic correlations were obtained between GRAM+ and AcM, ChM, CULL, and SCS ranging from 0.52 to 0.94, indicating that selection for these traits would reduce mastitis incidence and simultaneously improve SCC. The genetic relationship between the occurrence of infections with gram-positive bacteria seems to be particularly strong with traits that are indicators for persistent and poorly healing udder diseases (ChM, CULL). Gram-positive bacteria, such as *Staph. aureus*, can lead to chronic infections (Wellnitz and Bruckmaier, 2012), which can result in persistent intramammary infections (Sutra and Poutrel, 1994). Culling of infected cows therefore often remains the only viable option to limit the spread of infection in the herd. For GRAM−, genetic correlations were only found to be positive with AcM (0.48), which was also observed by Blowey and Edmondson (2010) at the phenotypic level.

Table 4. Estimated linear (h^2) and transformed threshold heritabilities (h^2_{th}) and standard errors (SE/SE_{th}) for gram-positive bacteria (Gram+), gram-negative bacteria (Gram−), bacterial infection (BACI), acute mastitis (AcM), chronic mastitis (ChM), culling due to udder health problems (CULL), and lactation mean SCS (LSCS) from multivariate analyses in Austrian Fleckvieh cows

Trait	h^2	SE	h^2_{th}	SE_{th}
5-variate analyses				
BACI	0.012	0.004	0.043	0.015
AcM	0.035	0.008	0.088	0.020
ChM	0.025	0.008	0.180	0.059
CULL	0.019	0.005	0.119	0.031
LSCS	0.197	0.024		
6-variate analyses				
Gram+	0.014	0.004	0.053	0.017
Gram−	0.012	0.005	0.134	0.057
AcM	0.035	0.008	0.089	0.021
ChM	0.022	0.007	0.158	0.049
CULL	0.018	0.004	0.117	0.027
LSCS	0.198	0.025		

The results suggest positive and strong genetic correlations between incidence of BACI and clinical mastitis based on veterinary diagnoses and culling data, as well as the mean SCS trait, ranging from 0.60 between BACI and AcM to 0.96 between BACI and CULL. As the correlations were lower than 1, the traits can be considered as partly different. As staphylococci and streptococci species were predominantly responsible for the infections, and their occurrence may cause acute as well as chronic mastitis, high correlations were expected.

In this study, genetic correlations between BACI, GRAM+, GRAM−, and LSCS were 0.88, 0.91, and <−0.001, respectively. Results show that through selection against high mean SCC, the incidence of udder infections would decrease, whereas this positive effect might not be true for infections with gram-negative bacteria. De Haas et al. (2002) reported weak to moderate genetic correlations in the range of 0.04 to 0.54 between different pathogen-specific mastitis traits and

Table 5. Genetic correlations and their SE (in parentheses) between bacterial infection (BACI), acute mastitis (AcM), chronic mastitis (ChM), culling due to udder health problems (CULL), and lactation mean SCS (LSCS) from multivariate analyses in Austrian Fleckvieh cows

Trait	AcM	ChM	CULL	LSCS
BACI	0.616 (0.160)	0.754 (0.137)	0.964 (0.069)	0.879 (0.109)
AcM		−0.041 (0.181)	0.540 (0.131)	0.528 (0.114)
ChM			0.806 (0.123)	0.608 (0.133)
CULL				0.725 (0.114)

lactation mean SCS in Dutch dairy cows. Moderate to high posterior means of genetic correlations (0.47 to 0.66) between pathogen-specific mastitis traits and lactation average SCC (5–300 DIM) were calculated in Danish Holsteins (Sørensen et al., 2009b).

Weak negative genetic correlations were obtained between AcM and ChM (−0.02 to −0.04), suggesting the traits to be completely different. Selection for higher resistance to acute mastitis might not increase resistance to chronic mastitis to the same extend. However, the large standard error has to be considered when interpreting this result.

Moderate genetic correlations were found between AcM and CULL, as well as LSCS and ChM and LSCS, in the range of 0.50 to 0.65. Koeck et al. (2010) reported a genetic correlation of 0.63 to 0.67 between lactation mean SCS and clinical mastitis traits in different lactation stages in Fleckvieh cows. Pritchard et al. (2013) obtained lower genetic correlations over the whole lactation, with estimates of 0.23 for SCS and clinical mastitis in Holstein cows from first to third parity.

Final Remarks

Although the veterinary diagnoses of mastitis in the current study were not standardized, but were made according to the clinical judgment of the respective herd veterinarians, in the authors' opinion this does not detract from the results analyzed here, as the data represent the practical real-world situation on farm.

Routines of performing bacteriological milk sampling depend on the motivation and strategy of the farmer. Increased testing will provide more pathogen-specific data to improve herd management and support targeted treatment of mastitis and dry-off strategies with potential to reduce the use of antimicrobials, as well as providing more information as a basis for genetic selection.

To give farmers the most comprehensive overview of udder health, all available udder health results should be stored in one database. In Austria, an interface between the laboratories and the central cattle database has been established, to allow the storage of standardized results of bacteriological analyses of quarter milk samples as additional information on udder health. The central data storage and its availability, as well as data analyses, enable proactive herd management and support farmers in their daily work. The central availability of all data also supports veterinarians in their diagnostic work.

CONCLUSIONS

Three traits were derived from results of bacteriological milk analyses, one covering all bacteriological infections and two with respect to groups of bacteria. The results showed potential to include the results of bacteriological milk analyses in genetic evaluations. Genetic correlations close to zero suggest that infections caused by gram-positive and gram-negative bacteria are not the same trait. Genetic correlations among infection traits and clinical mastitis and SCS traits were predominantly positive and strong but lower than 1. This indicates that these traits are different to some extent. Including pathogen information contributes to increased completeness of data and more targeted measures for improving udder health. Using pathogen

Table 6. Genetic correlations and their SE (in parentheses) between gram-positive bacteria (Gram+), gram-negative bacteria (Gram−), acute mastitis (AcM), chronic mastitis (ChM), culling due to udder health problems (CULL), and lactation mean SCS (LSCS) from multivariate analyses in Austrian Fleckvieh cows

Trait	GRAM−	AcM	ChM	CULL	LSCS
Gram+	−0.204 (0.271)	0.520 (0.132)	0.825 (0.108)	0.945 (0.082)	0.907 (0.070)
Gram−		0.479 (0.193)	−0.559 (0.214)	−0.235 (0.270)	<−0.001 (0.221)
AcM			−0.022 (0.199)	0.539 (0.153)	0.500 (0.117)
ChM				0.815 (0.120)	0.652 (0.118)
CULL					0.730 (0.121)

data as additional direct information may enable more efficient breeding for improved mastitis resistance in the future.

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