SOLE HAEMORRHAGE, DERMATITIS AND Co. - HOW GENOMIC INFORMATION AND PRECISE PHENOTYPES HELP TO UNSCRAMBLE GENETIC BACKGROUND OF HEALTH TRAITS IN DAIRY CATTLE

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SUMMARY

Disorders of the bovine hoof are important factors influencing the well-being and milk production of the dairy cow. Here, results from 2 different studies were used to demonstrate the value of how contemporary groups are defined, standardized recording, and improved trait definitions, to investigate the genetic background of important claw disorders. In the first study, 1,962 first-lactation cows from 7 commercial (contract) herds were subjected to hoof trimming with an assessment of hoof disorders as binary traits. Sole hemorrhage (SH), white line disease, sole ulcer, and interdigital hyperplasia (IH) showed to be the most important noninfectious claw disorders. The DNA of 1,183 of the cows was used for analyses with a custom-made array of 384 SNP. It revealed that SNP rs29017173 is significantly associated with SH disorder status. For IH, bull lineages with high proportions of IH affected daughters and granddaughters were identified. With the help of 192 genotyped cows (Illumina BovineSNP50 BeadChip) from well-selected cohorts, 4 candidate regions were identified. A second study based on 729 pregnant heifers from an US-American commercial dairy herd focused on bovine digital dermatitis (DD). New DD trait definitions were used to investigate the genetic background of this infectious disease. The new traits enabled the differentiation of clinical stages and their succession over time. The heritability estimates for the DD traits ranged between 0.19 and 0.52. An association study, based on 106 genotyped cows from this study, revealed 3 promising candidate regions.

INTRODUCTION

The implementation of genomic selection by large parts of the animal breeding industry has enabled gains in predicting the accuracy of breeding values, increased genetic progress, and allowed new breeding strategies. With more precise knowledge of the genetic information, it seems that the limitation is now phenotype availability. This limit is caused by either the quality or the quantity of phenotypes. In general, precise phenotypes and standardized data management are key aspects for high quality data. New or hard to measure traits often result in a small, or hardly representative sample. Health traits have recently been growing in importance, but are often especially challenging (Egger-Danner *et al.* 2015). Most farms record disease events as they occur and thus the successful principal to work with defined contemporary groups at a specific point in time is generally neglected.

This paper presents the use of defined contemporary groups, standardized recording, and improved trait definitions, to investigate the genetic background of and to identify associated genetic regions for the claw disorders sole hemorrhage, interdigital hyperplasia, and bovine digital dermatitis in dairy cattle.

MATERIALS AND METHODS

This paper is based on two different trials.

Trial_1: Seven commercial herds in north-eastern Germany were selected from a pool of contract dairy herds of the breed association Mecklenburg-Vorpommern. All herds had similar housing conditions and feeding. First lactation cows being in a similar stage of lactation were defined as contemporary groups. A fixed team of 3 people assessed a total of 1,962 first-lactation Holstein cows during 24 herd visits. Claw diagnoses were recorded for hind legs at the time of hoof trimming. The disorder status included assessing clinical and subclinical claw disorder cases as binary traits. A detailed description of the trial was described in Schöpke *et al.* (2013).

About half of the cows (1,183) were genotyped using a custom-made 384 array.

Before this study, no published QTL for sole hemorrhage (SH) were available. Thus, the selection of SNP for the custom-made array followed the general assumption that genetic correlation between leg conformation traits and claw disorders exists.

SNP selection was based on a 4-step-strategy that included an in silico analysis of published QTL associated with conformation traits in cattle and pigs; the identification of syntenic chromosomal regions across cattle, pigs, and human; the selection of candidate genes; and the selection and validation of SNP. The list of candidate genes contained 1,035 genes that were assigned to at least one of the biological functions development, function and disease of skeleton, muscles, and connective tissue; cell signalling; disorder in vitamin and mineral metabolism; or hydrate metabolism. For the array, SNP within 384 of these genes were selected according the following criteria: validation for NCBI or bovine 50K chip, SNP interval (0.7–1.0 Mb), minor allele frequency (>0.05), not located in a repeat region, and tested on a Holstein-Frisian population. With regard to the selection of cows for genotyping with the custom-made array, entire contemporary groups were selected that exhibit a "normal" range of prevalence rates for the claw disorder sole hemorrhage. All further samples were genotyped for 1 SNP that was strongly associated with SH by a fluorescence resonance energy transfer assay (Förster 1946). Detailed description for DNA extraction, SNP selection, and genotyping can be found in Swalve *et al.* (2014). In a second part of this trial, contemporary groups with the highest prevalence rates for interdigital hyperplasia (IH) were used as a basis for genotyping 192 cows with the Illumina BovineSNP50 BeadChip. as described in Sammler *et al*. (2015).

Trial_2: In this trial, 729 pregnant (nulliparous) heifers from a commercial Holstein dairy herd in Wisconsin, USA that was endemically affected by bovine digital dermatitis (DD), were inspected in a stand-up chute on a regular basis: at least 3 times per heifer within a mean (SD) individual cow observation time of 176 days (20.1). In total 6,444 clinical observations for DD were collected applying the M-score system as defined by Döpfer *et al.* (1997) and Gomez *et al.* (2014). This system is a classification scheme for stages of DD that allows a macroscopic scoring based on clinical inspections of the bovine foot, thus it describes the stages of lesion development. Briefly, lesions were classified as M0 for unaffected animals with no clinical lesions; as M1 for infected heifers with early lesions smaller than 2 cm in diameter (non-active); and as M2 for infected heifers with a classic active lesion of >2 cm of diameter considered to be infectious. An M4 stage denotes late and chronic stages of DD with (M4.1) or without (M4) small (<2 cm diameter) M1 lesions within their perimeter. M-scores were used to define new DD trait definitions with different complexity (Table 1) as described in Schöpke *et al.* (2015). Trait TBIN denoted a very basic description of the clinical DD status. This binary trait separated between unaffected heifers throughout the entire observation period and all other heifers. A special consideration of heifers reaching an active stage of lesion (M2 or M4.1) was given with trait TBINA. Trait TSEVCAT described the severity of DD cases a heifer was afflicted by in two slightly different versions. TSEVCAT was a categorical trait with 3 classes comparing not affected heifers (always M0; score = 1) with heifers having at least one M1, M4, or M4.1 but never M2 (score=2), and cows suffering at least once from classic active ulcers (M2; score=3). TSEVCAT41

was very similar to TSEVCAT but differed concerning heifers with at least one M4.1 event, which received a score of 3. The known difference between M2-cow types (Gomez *et al.* 2014) is basis for the definition of trait TCTM2SC that classified heifers according the number of active M2 lesions during the observation period (score of 1=type I heifer: no M2 lesions; score of 2=type II heifer: exactly one M2 lesion, score of 3=type III heifer: multiple M2 lesions). TCTM2SC counted all M2 events considering every leg separately and TCTM2 counted per event date. Trait TTRANS accounted for the changes of the M-stages over successive evaluations and thus included a better description of the DD infection dynamics. For TTRANS, the transitions between stages were classified (1: staying not-affected; 2: healing, improving; 3: staying affected on the same/comparable stage; 4: aggravating), the classes were weighted, and a transition score was derived. To compute TTRANS, for each heifer all observations were classified for heifer type as explained above, weighted, summarized, and divided by the number of transitions observed. A reference scenario (TREF) was defined by considering the first evaluation of each heifer as the only information. TREF denotes a single scoring for DD as has been commonly used in most studies applying genetic-statistical methods.

For genotyping, the Illumina BovineSNP50 BeadChip was used to genotype 63 animals; another 43 animals were genotyped with the BovineHD Genotyping Bead Chip (777K).

Table 1. Description of trait definitions for digital dermatitis, number of observations in the final data set, means, and standard deviations (SD); category frequencies instead of means for traits with more than two categories

Trait	Trait definition	No. of observations (no. of cows)	Mean	SD
TBIN	Binary trait that differentiates between consistently not DD affected cows (0) and cows with at least one observation with a DD lesion (1)	729 (729)	0.52	0.50
TBINA	Binary trait that differentiates between cows that never (0) / at least once (1) experience an active stage of DD lesion	729 (729)	0.40	0.49
TSEVCAT	Categorial trait that differentiates between three severity categories of DD lesions: consistently not affected (1), at least once M1, M4, or M4.1 (2), at least once M2 (3)	729 (729)	(1) 48.3% (2) 15.7% (3) 36.0%	
TSEVCAT41	Categorial trait that differentiates between three severity categories of DD lesions: consistently not affected (1), at least once M1 or M4 (2), at least once active stage M2 or M4.1 (3)	729 (729)	(1) 48.3% (2) 11.9% (3) 39.8%	
TCTM2	Categorial trait that differentiates between three DD cow types concerning the number of M2 events: never M2 (1), once M2, at least twice M2 (3)	691 (691)	(1) 64.0% (2) 14.5% (3) 21.5%	0.79
TCTM2SC	Categorial trait that differentiates between three DD cow types concerning the number of M2 events considering legs separately; never M2 (1), once M2, at least twice M2 (3)	691 (691)	(1) 64.0% (2) 18.4% (3) 17.6%	0.83
TTRANS	Transition score for the classified and weighted transitions between DD stages	729 (729)	17.90	9.05
TREF	Binary trait that differentiates the first observation of the cow into not affected (0) or affected (1)	729 (729)	0.12	0.33

Statistical Analyses: Data preparation, editing, and examination of alternative modelling of fixed effects as well as preliminary χ^2 tests for genotypic associations with SH, IH, or DD were conducted using the statistical package SAS 9.1 and 9.4. Variance components were estimated using a restricted maximum likelihood (REML) animal model and applying the ASReml 3.0 software package. Associations between genotype and disorder status were also tested using PLINK software.

RESULTS AND DISCUSSION

Trial_1: The 4 most important noninfectious disorders were sole hemorrhage, white line disease, sole ulcer, and interdigital hyperplasia with SH being the predominant disorder. Prevalence rate varied between 5.5 (IH) and 57.3 % (SH) showing remarkable differences of within-herd levels (Table 2). The prevalence level of SH is higher than in other studies, which is largely because "mild" or subclinical cases of diseases were included. For SH, herd-visit date, stage of lactation, and body weight significantly affected the probability of occurrence and thus were included as fixed effects in the model when accounting for SNP genotype effects. Analyses by PLINK of 295 SNP (MAF>0.5) revealed a highly significant association (P<0.001) between disorder status for SH and the SNP (HAPMap54883-rs29017173) within the IQGAP1 gene (BTA 21). The GLIMMIX analyses resulted in back-transformed means of the disorder status of 0.35 (AA), 0.49 (AG), and 0.54 (GG) when comparing the 3 genotypes in a reduced data set (herd-visit cohorts with extreme frequencies for SH were excluded). Using the full data set, the back transformed means of the SH status were 0.50 (AA), 0.56 (AG), and 0.60 (GG). Polymorphism of the SNP showed substantial effects for the occurrence of SH, but it was also found to be associated with feet and leg traits from the classical conformation score system. Fortunately, the same allele is favoured for all traits with substantial effects. IQGAP1 is proven via knock-out mice to play a critical role in postischemic neovascularization and tissue repair (Urao *et al.* 2010). Thus, it can serve as a promising candidate gene for the pathogenesis of SH in cattle. However, it might be more a question of tolerance here than of resistance.

When assessing the disorder status of interdigital hyperplasia (IH), 107 IH positive animals were identified. When separating the IH phenotype into one-side and pairwise affected rear legs, 71 cows and 36 cows were found, respectively. An investigation of sires with at least 5 daughters in the data set revealed an IH predisposition of 5 sires that are sons of the same bull. When comparing bulls with at least 140 granddaughters in the data set, the identified bull had a remarkably high proportion (9.4 %) of IH affected granddaughters. This result confirms the occurrence of IH in some bull lineages, this observation has occasionally been mentioned in literature (Hogreve 1964).

Table 2. Prevalence rates for the 4 most important noninfectious disorders and herd-visit prevalence interval

	All observations	Within herd - date of visit	
Name of disorder	[%]	Min [%]	Max [%]
Laminitis	57.3	25	92
White line disease	12.6	2	32
Sole ulcer	7.1	0	25
Interdigital hyperplasia	5.5	0	20

Within the 192 genotyped cows, 87 were IH positive (56 one-side affected vs. 31 pairwise affected) and 105 were negative (=controls). A case-control study on the genetic background of IH revealed associated regions on 4 different chromosomes.

Trial_2: From all 6,444 observations, 68.0 % of the records were found to be negative, i.e. "healthy" regarding DD. Out of the 32 % DD-positive observations 54.8 % showed a chronic stage (M4 or M4.1) of which 37.7 % were chronic and active (M4.1). The infectious stages M2 and M4.1 together accounted for 11.3 % of all observations. 48.2 % of the heifers were consistently not affected by DD during the entire observation time. Estimates for heritabilities from univariate models were 0.19±0.11 (TBIN), 0.20±0.11 (TBINA), 0.27±0.12 (TSEVCAT), 0.23±0.12 (TSEVCAT41), 0.46±0.16 (TCTM2SC), 0.52±0.17 (TCTM2), 0.42±0.15 (TTRANS), and 0.19±0.11 (TREF). Estimates of heritabilities for DD exist in the literature (e.g. van der Linde *et al*. 2010; Gernand and König 2014) however, none of the published studies used M-scale scored records. Estimates in the present study were higher than results from literature. This might be due to the limited sample size. Even though, the results presented are based on a well-established classification system for clinical stages and their succession over time, and thus serve as a comparison between trait definitions that reach beyond a separation of affected and non-affected animals. Those traits were also basis for the association study. Analyses by PLINK showed candidate regions on 3 different chromosomes. For 1 of the regions, a haplotype consisting of several SNP being in complete linkage disequilibrium was identified.

Conclusion. Phenotyping for health traits appears to be very difficult. This is partly due to dominant environmental effects and the occurrence of genotype by environmental interaction, but it is caused also by the difficulties of precisely defining "diseased" or "healthy" states. In Trial_1, an association between SH and the IQGAP1 gene was identified. The strength of this association differed between the full and the reduced data set, and thus it demonstrated that the detection of genetic effects or individual gene effects is strongly dependent on a well-planned study design, implying selection of herds, definition of phenotype, and time of evaluation. Trial_2 demonstrated the application of the M-scale scoring system when analysing the genetic pre-disposition for DD. This system is widespread in the veterinary field. Its first use for genetic analyses revealed higher heritability estimates than known from previous studies, and therefore the genetic predisposition for DD might be higher than previously assumed. The findings indicated that applying this improved phenotype definition of DD allows for improved management strategies and improved strategies for genetic selection.

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