Genomic analysis of claw lesions in Holstein cows: Opportunities for genomic selection, quantitative trait locus detection, and gene identification

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ABSTRACT

Claw lesions are the third most important health issue in dairy cattle, after mastitis and reproductive disorders, and genomic selection is a key component for long-term improvement of claw health. The objectives of this study were to assess the feasibility of a genomic evaluation for claw health in French Holstein cows, explore possibilities to increase evaluation accuracy, and gain a better understanding of the genetic determinism of claw health traits. The data set consisted of 48,685 trimmed Holstein cows, including 9,646 that were genotyped; 478 genotyped sires were also used. Seven claw lesion traits were evaluated using BLUP, genomic BLUP, BayesC, and single-step genomic BLUP, and the accuracies obtained using these approaches were measured through a validation study. The BayesC approach was used to detect quantitative trait locus (QTL) regions associated with the 7 individual traits (digital dermatitis, heel horn erosion, interdigital hyperplasia, sole hemorrhage circumscribed, sole hemorrhage diffused, sole ulcer, and white line fissure) based on their Bayes factor. Annotated genes on these regions were reported. Genomic evaluation approaches generally did not allow for greater accuracies than BLUP, except for single-step genomic BLUP. Accuracies were moderate, but best and worst validation animals were correctly discriminated and showed significant differences in lesion frequencies. A total of 192 QTL regions were identified, including 13 with major evidence or involved for 2 of the traits. A high number of genes were present on these regions, and several had functions associated with the immune system. In particular, the *EPYC* gene is located close to a major evidence QTL for resistance to digital dermatitis that is also a QTL for interdigital hy-

perplasia (on chromosome 5, around 20.9 MB) and has been associated with Ehlers-Danlos syndrome in cattle. Genomic selection can be used to improve resistance to individual claw lesions, and several possibilities exist to improve accuracies of genomic evaluations.

Key words: genomic evaluation, claw health, dairy cattle, quantitative trait loci

INTRODUCTION

Claw lesions are an issue in terms of both welfare (Bruijn et al., 2012) and profitability. They are ranked third in cost among health disorders in dairy cattle, after mastitis and reproductive disorders (Enting et al., 1997), mainly due to treatment costs, reduced milk production, discarded milk, increased calving interval, and increased involuntary culling (Bruijn et al., 2010; Charfeddine and P, 2017). Furthermore, claw lesions usually have quite high frequencies (Enting et al., 1997; van der Waaij et al., 2005; Charfeddine and P, 2017). In France, the proportion of trimmed cows with at least one lesion goes up to 82% (Crou et al., 2017).

Hence, it is crucial to reduce the frequency of claw lesions. Although heritabilities are often found to be very low (Heringstad et al., 2018), most studies conclude that there is enough genetic variability of claw health traits for genetic selection to occur and genetic selection is a key component for long-term improvement of claw health. This is why a few countries implemented genetic evaluations of claw health traits in recent years (Stoop et al., 2010; Johansson et al., 2011; Jamrozic et al., 2017) and others have ongoing projects with this goal (Gernand et al., 2012; Charfeddine and P, 2017). In France, a private company (EVOLUTION) has led a project for implementation of genetic evaluation of Holstein cows; the evaluation was released in autumn 2017 (EVOLUTION, 2017).

In addition to low heritabilities, claw health traits are generally difficult to measure and many sires only have

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a few number of daughters with records. Furthermore, in most cases, the start of data collection is recent, hence no historical data are available (Heringstad et al., 2018). In the context of dairy breeds with routine genomic evaluations for other traits, genomic selection is both needed and a challenge for these new traits.

The objectives of the present study were to assess the feasibility of a genomic evaluation for claw health in French Holstein cows, to explore possibilities to gain in accuracy, and to increase our knowledge on the genetic determinism of claw health traits.

MATERIALS AND METHODS

Data

The phenotypes used in this study were collected on French Holstein cows from April 2014 to December 2017, by claw trimmers trained with a unique protocol (France Génétique Elevage, 2013). These claw trimmers recorded observed claw lesions as they performed routine trimming in herds. Data were eliminated using the steps described in Croué et al. (2017), and minimal requirements of 100 cows trimmed per trimmer $\times$ year and 25 daughters per sire were added. Because most of the cows (86%) only had one record, only the first trimming information was considered for cows with multiple records.

The current study takes into account the phenotypes from 48,685 trimmed cows. Pedigree was traced back for 3 generations and includes 119,978 animals.

Traits

The traits considered are lesion traits with a binary definition: they were recorded as 1 if the trimmer observed the lesion during trimming and 0 if she did not. The lesions considered are digital dermatitis (**DD**), heel horn erosion (**HHE**), interdigital hyperplasia (**IH**), sole hemorrhage circumscribed (**SHC**), sole hemorrhage diffused (**SHD**), sole ulcer (**SU**), and white line fissure (**WLF**), with which white line abscess and double sole were grouped. These traits are mainly defined according to the International Committee for Animal Recording atlas (International Committee for Animal Recording, 2015) with some limited differences, as reported in Croué et al. (2017).

Genotypes

Genotypes were extracted from the French genomic database for cattle. Animals were genotyped (or imputed from low-density genotypes) on the Illumina Bo-

vine SNP50 BeadChip. Quality control was performed within breed. Genotyped animals with a call rate below 0.95 were removed from the analysis. The SNP showing departure from Hardy-Weinberg equilibrium ($P < 0.0001$) or with more than 10% missing genotypes were removed. Parent-progeny compatibility was verified and incompatible animals were eliminated. In the end, 43,801 SNP were used. For QTL detection, the custom part of the Illumina SNP50 BeadChip and EuroG10K was also considered, increasing the number of SNP used to 51,630. The supplementary SNP are specific SNP added on the chips for research purposes, with generally good annotations, and hence they are particularly interesting to study annotated genes on a specific region or the study of genetic defects. A total of 9,646 phenotyped cows and 478 sires had genotypes available. These sires had on average 102 daughters with phenotypes (25 to 1,225).

Evaluation Approaches

Three genomic evaluation approaches were considered and compared with a conventional BLUP: 2 2-step genomic methods, genomic BLUP (**GBLUP**; VanRaden, 2008) and BayesC (Habier et al., 2011), and single-step GBLUP (**SSGBLUP**; Misztal et al., 2009; Christensen and Lund, 2010), which combines pedigree and genomic information.

The BLUP and SSGBLUP were applied to the following single-trait animal model:

$$y_{ijk} = \mu + hv_i + ps_j + a_k + \varepsilon_{ijk},$$

where y_{ijk} is the observed phenotype for DD, HHE, IH, SHC, SHD, SU, or WLF; μ is the general mean; hv_i is the herd-date of visit contemporary group; ps_j is the effect of parity $\times$ lactation stage (1–50, 51–100, 101–150, 151–200, 201–250, 251–300, 301–350, 351–400, and 401–550 d); a_k is the additive genetic value of the animal treated as a random effect with a variance-covariance matrix proportional to the relationship matrix; and ε_{ijk} is a random residual, accounting for heterogeneous residual variances depending on a random trimmer $\times$ year effect. These heterogeneous residual variances were included in the model in the form of different weights given to records depending on the trimmer and year of trimming, estimated simultaneously with the effects in the model.

Single-step GBLUP is similar to a pedigree-based evaluation, with a relationship matrix combining pedigree and genomic information. The resulting inverse of the relationship matrix, $\mathbf{H}^{-1}$, is defined as (Aguilar et al., 2010):

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{pmatrix} 0 & 0 \\ 0 & \mathbf{G}^{*-1} - \mathbf{A}_{22}^{-1} \end{pmatrix},$$

where $\mathbf{A}^{-1}$ is the inverse of the pedigree relationship matrix, $\mathbf{A}_{22}^{-1}$ is the inverse of the pedigree relationship matrix among genotyped animals, $\mathbf{G}^{*-1}$ is the inverse of the genomic relationship matrix $\mathbf{G}^*$ ($\mathbf{G}^* = 0.95\mathbf{G} + 0.05\mathbf{A}_{22}$), with $\mathbf{G}$ constructed as in VanRaden (2008). $\mathbf{G}^*$ is scaled to $\mathbf{A}_{22}$, such that the means of the diagonal and off-diagonal elements of $\mathbf{G}^*$ equal the means of the diagonal and off-diagonal elements of $\mathbf{A}_{22}$, respectively.

Pre-adjusted performances were calculated from the BLUP official evaluation for these traits, in a multitrait context, for all individuals with a phenotype. For this step, cows sired by bulls with less than 25 daughters were also included and contemporary nontrimmed cows were considered, through the use of a “trimming status” trait as in scenario (3) of (Croué et al., 2017), using the model described in the present study for BLUP and SSGBLUP. For genomic approaches, these pre-adjusted performances were used to compute phenotypes for genotyped individuals. Phenotypes were either directly pre-adjusted performances (yield deviation; **YD**) for genotyped cows, or daughter yield deviations (**DYD**) for genotyped sires: the pre-adjusted performances of their nongenotyped daughters were corrected for their dam’s EBV and summarized into average progeny pre-adjusted performances. Both GBLUP and BayesC were run in a single-trait context for genotyped animals and their ancestors only. Both methods used YD and DYD as phenotypic records, weighted by their equivalent number of performances.

The BayesC and GBLUP approaches considered that part of the total genetic variance was represented by a residual polygenic effect; this part was estimated during the analysis. For BayesC, a fixed proportion π of 1% of the total SNP was considered to have an effect on the traits.

The BLUP and SSGBLUP were performed using the BLUPF90 software (Miształ, 2008) and GBLUP and BayesC using the GS3 software (Legarra et al., 2014).

The (co)variance components used for the present study were those estimated in Croué et al. (2017). Heritability estimates were very low to low: 0.02 for SHC and SHD, 0.05 for HHE and SU, 0.06 for WLF, and 0.09 for DD and IH.

Validation Study

To assess the predictive ability of the different evaluation approaches considered, a cross-validation study was implemented: all phenotypes collected on cows born after November 2013 were not considered in the

analyses, so that the 20% youngest genotyped animals had their phenotypes removed. These 2,025 cows were used as a validation population and mimicked young candidates to selection. On this validation population, 2 validation criteria were considered. Accuracy was assessed as the correlation between EBV and YD, divided by the square root of the heritability of the trait. Evaluation bias was assessed through the regression coefficient of YD on EBV. The closer the regression coefficient was to 1, the less biased the evaluation. Standard errors of bias and accuracy were estimated using bootstrap with 1,000 samples.

To further assess the ability to correctly rank selection candidates, the validation population was divided in 5 groups of equal size based on EBV, and the correlation between mean EBV and mean YD within group was calculated over the 5 groups. In addition, lesion frequencies in the best and worst groups were compared and a *t*-test was realized to determine whether the observed frequencies were significantly different.

QTL Detection

Phenotypes for QTL detection were YD and DYD, as described above, weighted according to their equivalent number of performances, using the whole reference population, including phenotypes of the validation population. A BayesC approach was used, assuming that 1% of the SNP had an effect at each iteration (i.e., 516 SNP were selected at each iteration). These SNP were assumed to capture all of the genetic variance.

The Bayes factor (**BF**; Schurink et al., 2012) was used to assess the degree of association between each SNP and the traits. The BF is defined as (Kass and Raftery, 1995):

$$\text{BF} = \frac{\frac{P_i}{1 - P_i}}{\frac{\pi}{1 - \pi}},$$

where P_i is the probability for the SNP to have a nonzero effect and π is the proportion of SNP having a nonzero effect. The Bayes factor is the criterion recommended for QTL detection when using Bayesian methods (Wakefield, 2009; Legarra et al., 2015).

The BF was transformed into 2logBF to gain clarity for visual appraisal of QTL. The SNP were regrouped in QTL regions as described in Michenet et al. (2016): QTL regions were identified around SNP having a 2logBF ≥ 6 (strong evidence). The SNP with 2logBF ≥ 2 (positive evidence) located close to the peak SNP were included in the QTL region when they were within a sliding window of 0.5Mb of the peak SNP. The window

Table 1. Lesion frequencies in the whole population, genotyped individuals, and validation individuals

Trait	Whole population	Genotyped individuals	Validation population
Digital dermatitis	0.30	0.26	0.25
Heel horn erosion	0.39	0.37	0.32
Interdigital hyperplasia	0.08	0.07	0.04
Sole hemorrhage circumscribed	0.13	0.13	0.18
Sole hemorrhage diffused	0.32	0.32	0.33
Sole ulcer	0.07	0.06	0.06
White line fissure	0.12	0.10	0.08

was slid when at least one SNP with $2\log BF \geq 2$ was integrated in the QTL region. The $2\log BF$ thresholds of significance were selected, as proposed by Kass and Raftery (1995).

Candidate genes associated with either major evidence QTL ($2\log BF \geq 10$) or overlapping QTL regions for 2 of the traits were identified from the Ensembl database, using Biomart (<http://www.ensembl.org/biomart/martview>). Genes were selected when they were located within 500 kb of the identified QTL regions. Mouse Genome Informatics (<http://www.informatics.jax.org/>) was then used to check the functions associated with each selected gene.

RESULTS

Lesion Frequencies

Lesion frequencies were generally high for HHE, DD, and SHD (Table 1). In general, genotyped individuals had lesion frequencies similar to or slightly lower than those of the whole population. The validation population (youngest genotyped animals) had generally slightly lower frequencies, except for SHC.

Genomic Evaluation

For all evaluation approaches, low to moderate accuracies were observed (Table 2). Regression coefficients

varied widely depending on the trait and evaluation approach. Standard errors were high, making the comparison between approaches difficult. Generally, genomic approaches allowed for slightly higher accuracies than BLUP. However, the difference was not significant for most of the traits. For the traits where these differences were significant (more than 1 SE difference between the 2 values), SSGBLUP was the approach allowing for the highest accuracy.

When grouping cows of the validation population into 5 groups depending on their EBV estimated using a SSGBLUP evaluation approach, we observed that, even for the traits with the lowest accuracy, the best and worst groups of animals were generally well discriminated (Figures 1 and 2). The best and worst groups based on EBV always corresponded to animals with on average good and bad preadjusted performances, respectively, although not all of the intermediary groups were properly ranked. For the traits with higher accuracy, group ranking was relevant for all groups (Figure 2).

The difference in lesion frequency among the worst and best groups were significant (with a first-order error of 10%) for all approaches and traits, except for SHC for all approaches and HHE when using BLUP (Table 3). Genomic approaches generally allowed for proper ranking of validation animals: validation animals with better EBV tended to be the less affected ones. For all traits, SSGBLUP was the approach allowing for the

Table 2. Accuracies (Acc.) and slopes of the regression of EBV on yield deviation for BLUP, genomic BLUP (GBLUP), BayesC, and single-step GBLUP (SSGBLUP), with SE in parentheses

Trait ¹	BLUP		GBLUP		BayesC		SSGBLUP	
	Acc.	Slope	Acc.	Slope	Acc.	Slope	Acc.	Slope
DD	0.25 (0.08)	0.57 (0.19)	0.27 (0.08)	0.44 (0.14)	0.27 (0.08)	0.35 (0.11)	0.29 (0.08)	0.47 (0.13)
HHE	0.28 (0.11)	0.88 (0.34)	0.42 (0.11)	0.80 (0.20)	0.39 (0.11)	0.55 (0.15)	0.51 (0.10)	1.11 (0.23)
IH	0.30 (0.08)	0.66 (0.19)	0.28 (0.08)	0.39 (0.11)	0.20 (0.08)	0.38 (0.12)	0.26 (0.08)	0.38 (0.12)
SHC	0.25 (0.14)	1.07 (0.61)	0.29 (0.14)	0.84 (0.41)	0.31 (0.14)	0.50 (0.23)	0.28 (0.14)	0.78 (0.40)
SHD	0.70 (0.13)	3.07 (0.59)	0.49 (0.13)	1.44 (0.39)	0.39 (0.14)	0.69 (0.25)	0.63 (0.14)	1.87 (0.41)
SU	0.44 (0.11)	1.08 (0.29)	0.51 (0.10)	0.96 (0.21)	0.50 (0.11)	0.72 (0.17)	0.50 (0.11)	0.91 (0.20)
WLF	0.33 (0.09)	0.86 (0.24)	0.45 (0.09)	0.83 (0.16)	0.47 (0.09)	0.65 (0.12)	0.53 (0.09)	0.96 (0.17)

¹DD = digital dermatitis; HHE = heel horn erosion; IH = interdigital hyperplasia; SHC = sole hemorrhage circumscribed; SHD = sole hemorrhage diffused; SU = sole ulcer; WLF = white line fissure.

Figure 1. Regression of mean yield deviation (YD) on mean EBV among 5 groups of validation animals, created according to their EBV, for the 2 traits with the lowest accuracy, using a single-step genomic BLUP (SSGBLUP) evaluation approach.

highest level of significance and hence the most consistent distinction between best and worst animals in terms of lesion frequencies.

QTL Detection

In total, 192 strong evidence QTL ($2\log BF \geq 6$) were identified for the 7 lesion traits. Detailed information (position of peak, $2\log BF$ of peak, beginning, and end of the QTL region) can be found for all QTL in Appendix Table A1. Quantitative trait loci were identified

Figure 2. Regression of mean yield deviation (YD) on mean EBV among 5 groups of validation animals, created according to their EBV, for the 2 traits with the highest accuracy, using a single-step genomic BLUP (SSGBLUP) evaluation approach.

for all traits: 41 were found for DD, 24 for HHE, 49 for IH, 9 for SHC, 7 for SHD, 27 for SU, and 35 for WLF. These QTL were spread all over the genome, with 1 to 11 QTL per chromosome for all traits. The QTL region sizes were on average 1.277 MB wide, ranging from a single SNP to 2.598 MB (for SU).

Eight of the QTL had major evidence ($2\log BF \geq 10$): 4 for IH, 2 for DD, and 1 for WLF and HHE (Table 4).

Six of the 192 QTL regions overlapped for 2 of the traits (Table 5): half of them involved DD and IH (chro-

Table 3. Difference in the observed frequency of lesions between the worst and best of the 5 groups of validation animals, based on EBV¹

Item	BLUP	GBLUP	BayesC	SSGBLUP
Digital dermatitis	0.07*	0.08**	0.09**	0.08**
Heel horn erosion	0.02	0.06†	0.06†	0.10**
Interdigital hyperplasia	0.03*	0.05**	0.04**	0.04**
Sole hemorrhage circumscribed	0.03	0.03	0.04	0.03
Sole hemorrhage diffused	0.13**	0.11**	0.07*	0.13****
Sole ulcer	0.05**	0.07****	0.06****	0.08****
White line fissure	0.05†	0.07**	0.07**	0.09****

¹GBLUP = genomic BLUP; SSGBLUP = single-step genomic BLUP.
Significance levels based on a *t*-test: † $P \leq 0.10$, * $P \leq 0.05$, ** $P \leq 0.01$, **** $P < 0.0001$.

Table 4. Major evidence ($2\log\text{BF} \geq 10$) QTL detected and number of annotated genes close to their regions

Trait ¹	Chromosome	Window start (MB)	Window end (MB)	Position of peak (MB)	$2\log\text{BF}^2$ at peak	No. of annotated genes
DD	2	83.349	83.349	83.349	11.5	1
DD	5	20.607	20.607	20.607	12.2	5
HHE	29	1.348	1.348	1.348	10.2	18
IH	3	15.600	15.600	15.600	10.4	38
IH	6	96.070	96.070	96.070	10.8	5
IH	8	98.811	98.848	98.811	10.3	3
IH	10	41.514	42.433	41.514	12.4	14
WLF	6	87.022	88.111	88.111	11.9	27

¹DD = digital dermatitis; HHE = heel horn erosion; IH = interdigital hyperplasia; WLF = white line fissure.

²BF = Bayes factor.

mosomes 1, 5, and 14), 1 DD and WLF on chromosome 19, 1 DD and SU on chromosome 20, and 1 IH and WLF on chromosome 14. The QTL region common to DD and IH on chromosome 5 is also a major evidence QTL for DD.

A quite large number of genes were annotated on or close to QTL regions identified above (either major evidence QTL or QTL involved in 2 traits): 38 for the IH QTL on chromosome 3, 17 for a region on chromosome 14, overlapping for IH and WLF. Details for all QTL regions can be found in Appendix Table A2.

DISCUSSION

Lesion Frequencies

Lesion frequencies were generally similar in the whole population and in the genotyped population: genotyped cows were a representative subset of the population, with consistent lesion frequencies, although they tended to have slightly fewer lesions. The youngest genotyped individuals had in general lower lesion frequencies, which was expected because younger cows are less likely to develop a lesion and some lesions very rarely occur early in life. This difference in frequencies causes validation animals to be not representative of the whole population. However, young candidate bulls

would be in a similar situation as their young daughters are less likely to develop lesions than older cows.

Genomic Evaluation

The development of genomic evaluations for claw health traits is still quite recent (Heringstad et al., 2018) and faces some specific challenges: generally low heritabilities, high costs of phenotyping, a low number of offspring with phenotype per sire and hence low accuracies of sire phenotypes (usually deregressed proofs or pre-adjusted performances of their daughters). Genomic evaluations are promising for the evaluation of claw health traits and can perform quite well. Given the challenges offered by these traits, it is necessary to assess the usefulness of a genomic evaluation in a new population and female reference populations are of major interest. Although the number of studies focusing on genomic evaluation of health traits is growing, few of them specifically focus on lameness (Parker Gaddis et al., 2014; McNeel et al., 2017), and only recently, some have focused on claw health (Dhakal et al., 2015; Ødegård et al., 2015; Stoop et al., 2015; Chesnais et al., 2016; Jamrozic et al., 2017; Naderi et al., 2018).

The definitions of evaluated traits vary widely among authors and they have various ways of estimating the accuracy of genomic evaluation approaches. Com-

Table 5. QTL regions common to 2 of the traits

Trait involved ¹	Chromosome	Start ²	End ²	Position of peak trait 1 (MB)	Position of peak trait 2 (MB)	$2\log\text{BF}^3$ peak trait 1	$2\log\text{BF}$ peak trait 2	No. of annotated genes
DD-IH	1	33.674	33.934	33.674	33.674	6.1	6.5	2
DD-IH	5	20.607	20.607	20.607	20.607	12.2	7.6	5
DD-IH	14	77.222	77.222	77.222	77.222	7.3	8.6	4
DD-WLF	19	30.413	30.479	30.341	30.479	6.8	7.0	10
DD-SU	20	47.920	47.920	47.920	48.511	6.9	9.1	1
IH-WLF	14	2.755	3.841	2.827	3.030	9.0	6.6	17

¹DD = digital dermatitis; IH = interdigital hyperplasia; SU = sole ulcer; WLF = white line fissure.

²Beginning and end of the overlapping region for traits 1 and 2.

³BF = Bayes factor.

pared with Naderi et al. (2018), where the definition of accuracy is similar to ours, our estimates are low. However, their design of the validation population was quite different, as they chose the optimal percentage of sick cows to allocate to the validation population to maximize accuracy, when in our case we focused on young individuals. Furthermore, they grouped various individual lesions under a general “claw disorders” trait where we evaluated individual lesion traits. Grouping the individual lesions under a single trait was used in most genomic studies focusing on claw health (Dhakal et al., 2015; Ødegård et al., 2015; Stoop et al., 2015; Chesnais et al., 2016), whereas in France it was decided to run genomic evaluation on individual lesion traits and then to summarize them in 2 selection indexes for selection purposes. These selection indexes are resistance to noninfectious lesions (10% SHC, 10% SHD, 40% SU, 40% WLF) and resistance to infectious lesions (50% DD, 25% HHE, 25% IH), weighted according to their importance for farmers. The very nature of the response variable (YD calculated from an animal linear model on binomial traits with low heritabilities and only one record per animal) might also be responsible for the generally low accuracies we observed, as the response variable has a low precision.

The standard errors of validation accuracy and bias estimates were high due to the moderate size of the female validation population and the low heritabilities of the traits considered. Generally, accuracy differences were not significant between evaluation approaches, genomic approaches did not necessarily perform better than regular BLUP, and ranking among BLUP, single, and 2-step evaluation approaches varied depending on the trait. However, when differences between evaluations were significant, SSGBLUP was the approach with the highest accuracy. Similar studies (Ødegård et al., 2015; Dhakal et al., 2015; Stoop et al., 2015; Naderi et al., 2018) found that genomic approaches led to greater accuracies than pedigree approaches and concluded that SSGBLUP was the best approach. However, these studies did not provide the standard errors of accuracy estimates or significance levels of differences in accuracies. Our study confirms that SSGBLUP is a relevant approach when a small female reference population is available, heritabilities are low, and only a small proportion of the individuals are genotyped. This is due to the exhaustive inclusion of information and transfer of information from genotyped to nongenotyped individuals.

Given the low accuracies observed, increasing the reference population should be a priority. As data collection is still quite recent in France, the number of phenotypes and genotypes is increasing rapidly and accuracy gains are expected in the months to come. Fur-

thermore, as the number of records collected increases, the percentage of cows with repeated claw trimmings also increases. Therefore, the evaluation model should be extended to include a permanent environment effect to take into account repeated records. This should also increase accuracy, as genotyped animals will have more accurate phenotypes.

As accuracies were low, 2 alternative validation procedures were considered to get a clearer view of the ranking of validation animals. This particular type of validation, although less precise, allows a reduction of the noise due to the low accuracy of the response variable for validation and is regularly used for the validation of health traits, with generally satisfying results (Malchiodi et al., 2015; McNeel et al., 2017). These alternative validation procedures allowed for a better understanding of the ranking of validation animals and confirmed that genomic evaluation of claw health traits can be used to generate genetic gain in our population. The ranking of validation animals was also consistent with the differences in frequency observed between the best and worst classes of validation individuals. Furthermore, the frequency differences confirmed that, despite the low estimates of heritabilities, variability for claw health traits exists in the population and selection is possible. Finally, this part of the study also confirmed that SSGBLUP appears to be the best approach among those studied as it allowed for the best segregation of healthy and affected cows.

QTL Detection

Detection of QTL revealed a large number of QTL with strong evidence, including 13 that were either major evidence QTL or QTL that were common for 2 of the traits, indicating they are less likely to be false positives. Seven of the 8 major QTL regions were QTL for an infectious traits and 3 of the 6 common QTL regions involved 2 infectious traits. One of these regions was a major QTL for DD and a region common for DD and IH. The common QTL regions for DD and IH might come from a common genetic background, as the genetic correlation between these traits is high (0.76, Croué et al., 2017) but could also be related to other factors. Interdigital hyperplasia can for instance be a consequence of an infection from DD or DD can occur on an IH, as the freshly grown flesh is more susceptible to infection and might be recorded as a DD even if it is not on its usual spot. Most of these 13 QTL regions overlapped or were close to several genes. This highlights the fact that resistance to claw lesions is related to a high number of genes with small effects, but also seems to be particularly driven by some specific chromosomal regions. It confirms that there is a genetic

determinism of claw health traits and that cows can be selected for specific resistance to claw lesions.

Some QTL detection studies focused on claw lesions specifically (Scholey et al., 2012; Swalve et al., 2014; van der Spek et al., 2015; Wu et al., 2016; Naderi et al., 2018) and others on feet and leg conformation (Buitenhuis et al., 2007; Cole et al., 2011; Wu et al., 2013). Once again, a large diversity of approaches and QTL selection criteria were used. However, 25 out of the 192 strong evidence QTL identified in our study were identical or close (less than 1MB apart) to a QTL reported in one of those studies. Five of them were located on chromosome 10 and 3 on chromosome 22. It would be interesting to further investigate the QTL detected in our study with a GWAS and compare the 2 studies. More specifically, given the data structure (low percentage of genotyped individuals) and the relatively low accuracy of pre-adjusted performances, using a SS-GWAS to combine pedigree, phenotype, and genotype information for QTL detection might be an effective approach.

The QTL regions identified for major evidence QTL and QTL common for 2 of the traits were generally dense in genes (145 annotated genes were located less than 500 kb apart from one of the QTL regions; among them 118 were located close to QTL regions for an infectious trait). Twenty-four of these genes, located on chromosomes 3, 5, 6, 8, 10, 14, and 19 had functions associated with the immune system, making them potential candidate genes for resistance to claw lesions; 20 of them were close to a QTL region for an infectious trait. In particular, *EPYC*, on chromosome 5 (20,909,663–20,950,211), close to a QTL identified for DD and IH, was associated with osteoarthritis in mice and, more interestingly, to a form of Ehlers-Danlos syndrome in cattle, which causes soft and hyper-extensible skin, delayed wound healing, and skin fragility (Tajima et al., 1999). This might explain a higher susceptibility to DD and a longer time before healing.

Except for *EPYC*, no other obvious candidate genes were observed for resistance to claw lesions. However, a few promising QTL (either with major evidence or overlapping for 2 of the traits) exist, and including them in a genetic evaluation model, either through a marker-assisted approach or weighting SNP depending on their influence on the traits, might allow for extra gains in accuracy.

CONCLUSIONS

Genomic selection is a promising tool to increase resistance to claw lesions in French Holstein cows. Although validation accuracies were quite low and heritabilities are very low, groups of best and worst

validation animals are well discriminated and enough variability is present for genetic improvement to occur. The QTL detection study also confirms the existence of a genetic determinism of these traits. Several levers can be used to increase the accuracy of the genomic evaluation of claw health: an obvious increase of the reference population, the use of a SSGBLUP approach, and the inclusion of specific QTL in the evaluation model. A lot of genes are located in the QTL, including some potential candidate genes that could be further investigated; *EPYC*, located on chromosome 5, is the most promising one.

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APPENDIX

Table A1. Full list of identified QTL¹

Trait	Chromosome	Window start (MB)	Window end (MB)	Position of peak (MB)	2logBF at peak ²
IH	1	33.2841	33.9338	33.6739	6.5
DD	1	33.6739	33.9338	33.6739	6.1
WLF	1	38.625	38.8117	38.625	7.8
WLF	1	39.6929	39.6929	39.6929	7.2
DD	1	67.4442	67.4917	67.4917	6.5
IH	1	95.3436	95.3949	95.3436	6
SU	1	109.707	111.06	110.721	7.7
SU	1	119.329	119.447	119.344	6.9
HHE	1	120.338	120.479	120.338	8.4
WLF	1	128.373	128.373	128.373	6.5
SHC	1	136.868	136.868	136.868	6.1
SHC	1	158.162	158.162	158.162	7.4
SHC	2	3.15199	3.67329	3.4078	6.8
WLF	2	57.4811	57.7272	57.4811	6.4
HHE	2	67.5752	67.7661	67.7661	7.1
DD	2	83.3491	83.3491	83.3491	11.5
IH	2	85.3623	85.4521	85.3623	8
WLF	2	95.0693	95.2459	95.2459	6.5
WLF	2	102.906	103.282	102.906	7.8
DD	2	109.501	109.501	109.501	6.4
IH	2	114.775	115.715	115.715	6.6
DD	3	9.97418	10.6404	10.4625	6.8
IH	3	15.6001	15.6001	15.6001	10.4
SU	3	15.728	16.2657	16.1521	7.2
HHE	3	44.9015	44.9015	44.9015	6.2
SU	3	98.3162	98.5216	98.4121	6
IH	3	100.127	101.165	100.229	6
HHE	3	109.016	109.494	109.016	7.2
DD	3	113.354	114.284	114.284	6.2
HHE	4	1.9444	1.9444	1.9444	7.5
IH	4	7.33837	7.82371	7.33837	6.8
HHE	4	17.5563	17.5563	17.5563	6.2
SU	4	28.1172	28.6216	28.6216	7.7
WLF	4	67.6545	67.7728	67.7728	8.9
DD	4	73.8615	73.8615	73.8615	6.1
DD	4	82.0029	82.0738	82.0029	6.7
SU	4	118.934	120.532	120.336	6.8
IH	5	7.62558	7.62558	7.62558	7.6
SHC	5	8.51867	8.68361	8.56267	7
DD	5	20.6099	20.6099	20.6099	12.2
IH	5	20.6099	20.6099	20.6099	7.6
HHE	5	27.8396	28.8597	28.6951	7
IH	5	40.7628	40.7628	40.7628	6.5
SHD	6	53.9157	54.1372	54.1372	6.2
WLF	6	87.0221	88.1112	88.1112	11.9
SU	6	88.4813	89.049	88.8223	6.1
IH	6	96.0704	96.0704	96.0704	10.8
IH	6	103.752	104.197	104.197	7.1
WLF	7	0.86388	1.65698	1.65698	6.1
DD	7	17.404	17.404	17.404	7.4
SU	7	36.555	36.555	36.555	6.4
IH	7	53.3145	53.513	53.336	6.5
WLF	7	56.3317	56.3317	56.3317	6.2
DD	7	108.326	108.964	108.964	6.3
SHC	7	109.596	109.634	109.596	6.9
DD	8	1.76792	1.79445	1.79445	6.7
WLF	8	6.31171	6.37288	6.37288	6.7
HHE	8	30.2848	30.3206	30.3206	6.7
HHE	8	34.5845	34.9476	34.9476	6
WLF	8	36.4899	36.4899	36.4899	8.7
DD	8	36.6419	37.1582	37.1566	7.4
IH	8	71.6003	71.6003	71.6003	6.1
IH	8	98.811	98.848	98.811	10.3
SHD	8	102.859	103.61	103.484	7.5

Continued

Table A1 (Continued). Full list of identified QTL¹

Trait	Chromosome	Window start (MB)	Window end (MB)	Position of peak (MB)	2logBF at peak ²
HHE	9	16.835	16.835	16.835	6
SU	9	23.7667	23.785	23.785	6.7
DD	9	27.6164	27.6841	27.6841	8.4
IH	9	36.4403	36.4403	36.4403	6.9
IH	9	63.7723	64.23	63.7723	6.3
DD	9	80.6341	80.6341	80.6341	9.1
IH	9	93.2462	93.2462	93.2462	6.7
IH	9	97.7605	98.0945	97.7909	7.3
IH	10	4.61037	4.61037	4.61037	6.3
SU	10	6.87121	7.00566	7.00566	6.4
DD	10	26.6147	26.6406	26.6147	6.7
IH	10	41.5142	42.4327	41.5142	12.4
DD	10	47.9244	47.9244	47.9244	7
SU	10	51.0129	51.1559	51.1559	7.8
WLF	10	54.0129	54.0129	54.0129	6.2
DD	10	90.747	90.747	90.747	6.9
IH	11	2.11925	2.11925	2.11925	8.2
SHD	11	6.75849	6.75849	6.75849	6.2
HHE	11	8.48276	8.48276	8.48276	6.9
IH	11	10.0432	10.6305	10.1956	7
HHE	11	14.5234	14.7949	14.5234	7.6
HHE	11	23.4021	23.4021	23.4021	7
IH	11	27.5196	27.6934	27.6707	7.6
SHC	11	64.845	64.9067	64.9067	6.7
SU	12	11.6761	12.141	12.141	6.3
SU	12	54.1844	54.3252	54.3252	6.8
HHE	12	85.569	85.569	85.569	7.1
DD	12	89.1177	89.1177	89.1177	6.5
DD	13	15.7767	15.8993	15.7767	6.7
IH	13	35.534	35.6145	35.534	6.1
IH	13	49.9636	50.0043	50.0043	6.8
SU	13	53.1218	53.1218	53.1218	6.1
DD	13	66.9922	67.1221	66.9922	7
DD	13	69.6706	69.6706	69.6706	6.8
DD	13	71.2123	71.819	71.819	6.6
WLF	13	82.1768	82.6213	82.6213	6.5
WLF	14	2.64161	4.12741	3.03	6.6
IH	14	2.75491	3.84058	2.82663	9
IH	14	17.0025	17.6147	17.5179	7.7
SU	14	44.9854	45.004	44.9854	6.8
IH	14	60.5952	60.5952	60.5952	6.6
SHD	14	66.3393	66.3393	66.3393	6.1
IH	14	70.2506	71.0967	70.5702	6.8
DD	14	75.9915	77.222	77.222	7.3
IH	14	77.222	77.2539	77.222	8.6
IH	14	80.5435	80.5583	80.5435	6.4
SU	15	2.31712	2.34944	2.31712	6.3
IH	15	34.5392	34.8527	34.8527	6.7
HHE	15	37.8567	38.0031	37.893	6.2
HHE	15	41.9268	42.1751	41.9268	8.2
IH	15	54.1279	54.1279	54.1279	7
SHD	15	56.5484	57.154	56.5484	6.1
WLF	15	67.0621	67.0621	67.0621	6.8
WLF	15	70.6863	70.8504	70.824	6.2
IH	16	20.4723	20.5215	20.5215	6.1
HHE	16	43.6423	43.7424	43.6423	6.7
IH	16	51.2999	51.6218	51.6218	6.4
WLF	16	57.8885	57.8885	57.8885	6.1
WLF	16	63.94	65.0056	63.94	8.9
HHE	17	5.59097	5.59097	5.59097	6.7
DD	17	8.33105	8.41165	8.33105	9.1
SU	17	10.2501	10.4989	10.2501	9.4
WLF	17	23.3932	23.3932	23.3932	6.7
IH	17	31.4474	32.2697	32.2697	6.3
DD	17	33.338	34.2417	33.4956	7.7
SU	17	61.6763	61.7171	61.6763	8.3

Continued

Table A1 (Continued). Full list of identified QTL¹

Trait	Chromosome	Window start (MB)	Window end (MB)	Position of peak (MB)	2logBF at peak ²
SHC	17	74.3936	74.3936	74.3936	8.5
HHE	18	4.72391	4.8201	4.72391	6.4
HHE	18	22.0641	22.3001	22.189	6.4
WLF	18	34.5054	34.6663	34.6663	6.3
WLF	18	35.8299	36.2398	35.8299	7.2
IH	18	40.8161	40.8433	40.8433	7.8
WLF	18	42.7502	43.3792	42.8566	6.7
SU	18	58.142	58.6663	58.404	6.9
IH	18	63.6722	63.6754	63.6728	7.1
IH	19	28.5459	28.5459	28.5459	6.5
DD	19	30.3407	31.0332	30.3407	6.8
WLF	19	30.4133	30.4791	30.4791	7
SU	19	50.2959	50.505	50.4212	6.2
WLF	20	5.63774	5.63774	5.63774	6.3
SU	20	47.5707	48.5107	48.5107	9.1
DD	20	47.9197	47.9197	47.9197	6.9
IH	20	67.6208	67.82	67.6208	6.3
HHE	21	2.46122	2.69016	2.6429	6.6
DD	21	17.7124	17.7124	17.7124	6.6
DD	21	37.9859	39.1841	38.0248	6.2
DD	21	39.7163	39.7163	39.7163	6.6
DD	21	62.4022	62.4022	62.4022	7.4
DD	22	6.06425	6.53912	6.19086	6.9
WLF	22	32.2088	32.3248	32.3248	6.4
SU	22	32.6939	32.6939	32.6939	6
SHD	22	46.58	46.851	46.851	6.1
IH	22	52.8993	52.8993	52.8993	7.3
WLF	22	56.0855	56.0855	56.0855	6
DD	23	21.3318	22.2405	21.3318	6.6
WLF	23	40.9258	41.4571	41.3495	6.3
WLF	23	44.5772	45.3936	45.3936	6.1
IH	23	50.6882	51.4564	50.9712	6.1
SU	24	19.6561	20.0583	20.0371	6.3
SU	24	31.8863	32.1714	32.1714	6.5
DD	24	58.3274	58.5766	58.5766	6.8
WLF	24	58.6356	58.6356	58.6356	6.6
DD	24	61.9319	61.9319	61.9319	7.3
DD	25	5.728	6.05079	5.728	7.5
IH	25	11.7608	12.3592	11.7608	6.1
DD	25	22.438	22.5916	22.438	7.6
IH	25	27.2304	27.2304	27.2304	6.4
SHC	25	31.0567	31.1478	31.1478	7.3
WLF	25	34.7681	34.9641	34.7681	6.5
SHC	25	36.2015	36.6045	36.2015	6.6
IH	26	8.22127	9.00466	8.53981	6.3
IH	26	29.6588	30.0637	29.776	6.3
WLF	26	45.3434	46.0283	45.3434	7.7
WLF	27	31.6959	32.2542	31.7577	7.7
HHE	27	35.6414	36.2124	35.6414	6.6
WLF	28	15.1679	16.1714	15.5549	7.8
DD	28	22.5352	22.5352	22.5352	8.2
SU	28	25.0407	25.1754	25.0895	6.1
SU	28	25.7716	26.0352	26.0352	6.7
SU	28	26.7563	27.0721	26.7861	6.5
WLF	28	36.0582	36.4106	36.4106	6.2
SHD	28	37.7998	37.7998	37.7998	6.7
HHE	29	1.34847	1.34847	1.34847	10.2
IH	29	5.26585	5.26585	5.26585	6.8
IH	29	14.5781	14.5781	14.5781	7.5
HHE	29	16.7946	16.8872	16.7946	6.9
DD	29	16.9285	18.0905	17.4607	6.1
DD	29	50.6899	50.6899	50.6899	9.1

¹SHC = sole hemorrhage circumscribed; SHD = sole hemorrhage diffused; DD = digital dermatitis; HHE = heel horn erosion; IH = interdigital hyperplasia; SU = sole ulcer; WLF = white line fissure.

²BF = Bayes factor.

Table A2. Annotated genes on all major evidence QTL and QTL common for 2 traits; in bold: genes associated with immune system functions

Chromosome	QTL region start (MB)	QTL region end (MB)	Annotated genes
1	33.674	33.934	<i>CADM2</i> + 1 unnamed
2	83.349	83.349	1 unnamed
3	15.600	15.600	<i>DAP3</i> , <i>ASH1L</i> , <i>FDPS</i> , <i>PKLR</i> , <i>HCN3</i> , <i>CLK2</i> , <i>SCAMP3</i> , <i>FAM189B</i> , <i>GBA</i> , <i>MTX1</i> , <i>THBS3</i> , <i>bta-mir-92b</i> , <i>MUC1</i> , <i>TRIM46</i> , <i>KRTCAP2</i> , <i>DPM3</i> , <i>SLC50A1</i> , <i>EFNA1</i> , <i>EFNA3</i> , <i>EFNA4</i> , <i>ADAM15</i> , <i>DCST1</i> , <i>DCST2</i> , <i>ZBTB7B</i> , <i>LENEP</i> , <i>FLAD1</i> , <i>CKS1B</i> , <i>SHC1</i> , <i>PYGO2</i> , <i>PBXIP1</i> , <i>RF00432</i> , <i>PMVK</i> , <i>RF00026</i> , <i>KCNN3</i> , <i>ADAR</i> , <i>CHRNA2</i> , <i>UBE2Q1</i> , <i>TDRD10</i>
5	20.607	20.607	<i>CCER1</i> , <i>EPYC</i> , <i>KERA</i> , <i>LUM</i> , <i>DCN</i>
6	96.070	96.070	<i>NAA11</i> , <i>GK2</i> , <i>RF00026</i> , <i>ANTXR2</i> , + 1 unnamed
6	87.022	88.111	<i>UGT2A1</i> , <i>SULT1B1</i> , <i>SULT1E1</i> , <i>CSN1S1</i> , <i>CSN2</i> , <i>HSTN</i> , <i>STATH</i> , <i>CSN1S2</i> , <i>ODAM</i> , <i>CSN3</i> , <i>AMTN</i> , <i>AMBN</i> , <i>ENAM</i> , <i>JCHAIN</i> , <i>UTP3</i> , <i>RUFY3</i> , <i>GRSF1</i> , <i>MOB1B</i> , <i>DCK</i> , <i>SLC4A4</i> , + 7 unnamed
8	98.811	98.848	<i>ZNF462</i> , <i>RAD23B</i> , <i>KLF4</i>
10	41.514	42.433	<i>RF00017</i> , <i>LRR1</i> , <i>RP36AL</i> , <i>MGAT2</i> , <i>DNAAF2</i> , <i>POLE2</i> , <i>KLHDC1</i> , <i>KLHDC2</i> , <i>RF00614</i> , <i>NEMF</i> , <i>RF00017</i> , <i>RF00026</i> , + 2 unnamed
14	2.755	3.841	<i>SCRIB</i> , <i>FAM83H</i> , <i>MAPK15</i> , <i>CCDC166</i> , <i>ZNF623</i> , <i>TSTA3</i> , <i>PYCR3</i> , <i>TIGD5</i> , <i>EEF1D</i> , <i>NAPRT</i> , <i>MROH6</i> , <i>GSDMD</i> , <i>ZC3H3</i> , <i>MAFA</i> , + 3 unnamed
14	77.222	77.222	<i>MMP16</i> , <i>SLC2A5</i> , + 2 unnamed
19	30.413	30.479	<i>MYH8</i> , <i>MYH4</i> , <i>MYH1</i> , <i>MYH2</i> , <i>MYH3</i> , <i>SCO1</i> , <i>ADPRM</i> , <i>TMEM220</i> , + 2 unnamed
20	47.920	47.920	1 unnamed
29	1.348	1.348	<i>VSTM5</i> , <i>MED17</i> , <i>C29H11orf54</i> , <i>TAF1D</i> , <i>RF00561</i> , <i>RF00425</i> , <i>RF01161</i> , <i>RF00393</i> , <i>RF00408</i> , <i>RF00342</i> , <i>RF00421</i> , <i>RF004023</i> , <i>CEP295</i> , <i>RF00493</i> , <i>RF004945</i> , <i>SMCO4</i> , <i>DEUP1</i> , <i>SLC36A4</i>