

Genetic background of claw health in dairy cattle

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Genetic background of claw health in dairy cattle

D. van der Spek

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Abstract

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Claw disorders affect cow welfare and profitability of farms and as such are important traits relevant to dairy cattle breeding. Aim of this thesis was to increase our understanding of the genetic background of claw disorders to enable selection for reduced claw disorder incidence. The claw disorders were: abscess, corkscrew claw, (inter-)digital dermatitis or heel erosion (DER), double sole (DS), hardship groove, interdigital hyperplasia (IH), interdigital phlegmon, sand crack, super-foul, sole hemorrhage (SH), sole injury, sole ulcer (SU), white line separation (WLS), and yellow discoloration of the sole. Data was collected on Holstein cows kept in dairy herds in France. Individual claw disorder frequencies ranged from 0.1% to 23.8% and more than half of the trimmed cows had at least one claw disorder in at least one hind leg between 2007 and 2012. Heritabilities were estimated for DER, DS, IH, SH, SU, and WLS, and ranged from 0.02 to 0.14. Repeatabilities ranged from 0.02 to 0.33. The need for trimming ("trimming status") was found to be heritable as well with a heritability of 0.09. A high need for trimming the claws of cows is unfavorable and therefore trimming status is an interesting trait to include in genetic evaluation. Most claw health traits had similar heritabilities and were genetically the same trait in different parities, lactation stages and herds with different trait frequencies. Claw disorder frequency in Montbeliarde cows ranged from 9.4% to 41.1% and 73% of the cows had at least one claw disorder in at least one hind leg between 2007 and 2013. Heritabilities ranged from 0.01 to 0.09. Heritability for trimming status was 0.06, confirming that trimming status is a heritable trait.

To identify genomic regions associated with claw disorders and trimming status, a genome wide association study was performed. In total, 11 significant and 46 suggestive SNP were detected. Three of the suggestive SNP could be validated using a dataset of genotyped bulls. The detected SNP were spread across the genome and a major gene was not found.

In the general discussion, alternative ways of measuring claw disorders were discussed. Accuracy of progeny testing and genomic selection for claw disorders was compared and a breeding program to reduce claw disorders was simulated in order to estimate selection response. Reducing the incidence of claw disorders is achievable with selection, but at a cost of a decrease in production.

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1

General introduction

1.1 Introduction

Claw disorders in dairy cattle cause economic losses and welfare problems (Enting et al., 1997). Economic losses are caused by reduced milk production, increased involuntary culling, prolonged calving interval, treatment by a veterinarian or claw trimmer, and increased labor for the farmer (Collick et al. 1989; Barkema et al. 1994; Green et al. 2002; König et al. 2005; Bruijnis et al. 2010). The prevalence of different claw disorders in European dairy cattle housed in free-stalls is often more than 70% (Van der Linde et al., 2010; Fjeldaas et al., 2006; Van der Waaij et al., 2005; Manske et al., 2002; Philipot et al., 1994; Smits et al., 1992), meaning that over 70% of the cows in a herd have at least one claw disorder in the measured time period. Prevalences depend upon herd type as prevalences below 50% were found in dairy cattle kept in tie-stalls, beef-cow herds and dairy cattle kept in organic herds (Fjeldaas, 2006, 2007; Vaarst et al., 1998). Frequencies of different recorded claw disorders vary widely from 0.6% to 39.9% with heel horn erosion and sole hemorrhages having the highest frequencies (Van der Linde et al., 2010; Kujala et al., 2009, 2010; Bicalho et al., 2009; Capion et al., 2009; Holzhauer et al., 2008; König et al., 2005; Sogstad et al., 2005b; Somers et al., 2005; Van der Waaij et al., 2005; Manske et al., 2002). The most commonly reported claw disorders are different types of dermatitis, heel horn erosion, sole ulcers, hemorrhages and different types of white-line disorders.

Claw disorders can be divided into different categories according to their etiology, these are metabolic, infectious and traumatic lesions. Infectious or partly infectious lesions are digital dermatitis, interdigital dermatitis, interdigital phlegmon, and heel horn erosion. Metabolic or laminitis related hoof lesions are hemorrhages, sole ulcers, double soles, underrun soles, and white line problems. Traumatic lesions are for example excessive abrasion of the sole or injuries from e.g. small sharp stones, glass, or nails. Figure 1.1 shows different zones of the claw. White line problems often occur in zone 1, 2 and 3. Zone 4, 5, and 6 are typical areas for hemorrhages and ulcers. Heel erosion occurs typically in zone 6 and digital dermatitis in zone 7. Interdigital dermatitis and interdigital hyperplasia typically occur in zone 0. Figure 1.2 to 1.7 show examples of claw disorders often recorded in dairy cattle.

1.2 Risk factors

Many risk factors predisposing claw disorders have been found, among them are herd management factors as husbandry, housing and nutrition and individual cow factors. For example, Philipot et al. (1994) found an increased risk for claw disorders in herds with poor hygiene. Somers et al. (2005) found that restricted grazing time increased the risk for digital dermatitis and Barker et al. (2009) found increased risk for claw disorders in larger herds. Also nutrition was associated with the prevalence of claw disorders (Toussaint-Raven, 1985). Somers et al. (2005a,b) found that an increased concentrate amount after calving and feeding by-products increased the risk for claw disorders and Faye and Lescourret (1989) found that feeding maize silage and mineral supplementation increased the risk for claw disorders. Parity and stage of lactation of the cow had an influence on the occurrence of claw disorders. For increasing parities and during the peak of lactation an increased risk was found for most claw disorders (Enevoldsen et al. 1991a,b; König et al. 2005; Sogstad, 2005a, 2007; Chapinal et al. 2010; Kujala et al. 2010). Also a higher milk production was found to be associated with increased incidence of claw disorders (Dohoo and Martin, 1984; Lucey et al., 1986; Hultgren et al., 2004). Holstein cows were at higher risk compared to Meuse Rhine Yssel (Holzhauer et al. 2006), Norwegian cattle (Baird et al. 2009) and Ayrshire cows (Kujala et al. 2009). These breed differences suggest a genetic background for claw health.

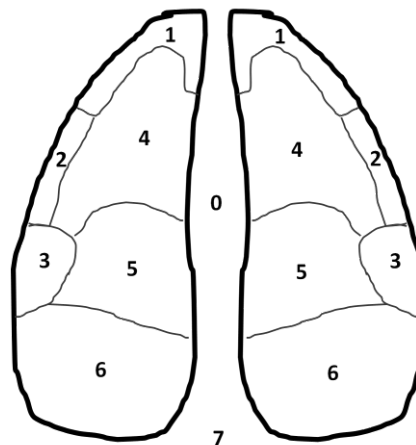


Figure 1.1. Numbering system to designate specific claw zones for recording claw disorders (based on Amstel and Shearer, 2006).



Figure 1.2. Heel horn erosion.
Degrading heel bulb, dark V-shaped
grooves.



Figure 1.3. Digital dermatitis or
Mortellaro disease.
Skin infection above the heel bulb visible
as a red growth.



Figure 1.4. Interdigital hyperplasia.
Growth of tissue between the claws.



Figure 1.5. Sole ulcer.
Infection of the corium, tissue protruding the sole.

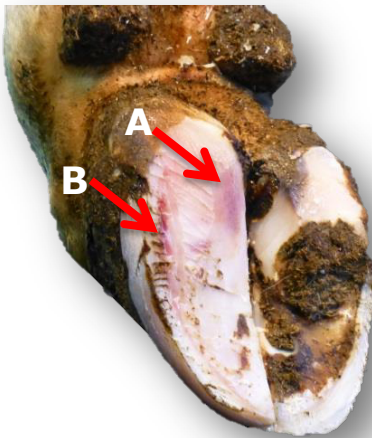


Figure 1.6. Hemorrhage of the sole (A) and white line (B).
Bruising and red coloration.



Figure 1.7. White line separation.
A small separation between the sole and the sole wall.

1.3 Selection for reduced susceptibility to claw disorders

Genetic selection provides a means to improve claw health, but requires genetic variation. Genetic parameters of extensive claw disorders scored by claw trimmers have been estimated previously. Heritabilities range from 0.01 to 0.23 (Van der Linde et al., 2010; Harder et al., 2006; König et al., 2005; Van der Waaij et al., 2005; Huang and Shanks, 1995; Smit et al., 1986). Not all cows in the herds are trimmed but a preselection is made. Therefore, in some studies it was decided to select herds where at least half (Van der Linde et al., 2010) or 75% of the cows were trimmed (Van der Waaij et al., 2005).

Estimation of breeding values is based on phenotypic records and pedigree information. Claw disorders have a low heritability and therefore many records of relatives are needed to accurately estimate the breeding value of an individual. The use of DNA marker information can help to accurately estimate breeding values early in the animal's life and is enabled with the emergence of new genotyping technologies. Different methods are available for including marker information in breeding value estimation. One approach is to use information from known genes or chromosomal regions and another approach is genomic selection. With linkage analysis or association studies (candidate) genes can be identified and these genes can be used in the breeding value estimation. Only markers with large effects are expected to be significant (Hayes and Goddard, 2010; Georges et al., 1995). Several production and health related traits have been shown to be affected by many genes each with a small effect (Cole et al. 2009; Hayes and Goddard, 2001; Klungland et al., 2001), these genes might not be significant in linkage analysis or association studies. With genomic selection, all (instead of a selected set of) markers are used to estimate breeding values (Meuwissen et al., 2001). Due to the availability of many markers and high throughput genotyping technology, genomic selection has recently become available. With DNA marker information an equation can be derived to predict genomic estimated breeding values (GEBV). Compared to traditional selection, genomic selection is considerably more accurate for animals without own performance records and especially for low heritable traits (Calus et al., 2008). Claw disorders in dairy cattle seem to have a low to moderate heritability and therefore genomic selection is a promising method to reduce susceptibility to claw disorders.

1.4 Aim and outline of this thesis

The objective of this thesis is to increase our understanding of the genetic background of claw disorders in dairy cattle to enable selection for reduced incidence of claw disorders.

For genetic selection to be successful, genetic variation is essential. In chapter 2, variance components, heritabilities, repeatabilities, genetic and phenotypic correlations among claw disorders were estimated for Holstein Friesian cows. Besides, the effect of trimming only a preselected proportion of the cows in the herd on genetic parameters was investigated.

Genomic regions associated with claw disorders may be used in genetic selection against claw disorders. To our knowledge, no genome-wide association studies have been published on the different claw disorders in dairy cattle. Information on genes involved in the development of claw disorders would be valuable for a better understanding of the development of claw disorders and to include this information in the breeding value estimation for dairy cattle. In chapter 3 a genome-wide association study was performed to investigate if major associations of markers or genes with claw disorders can be identified.

In order to gain more insight in the genetic background of claw disorders, it was investigated in chapter 4 whether claw disorders are genetically the same trait in different parities, lactation stages, and herds with different claw disorder frequencies.

Genetic background of claw disorders in a different breed, the Montbeliarde, have not been published yet, to our knowledge. The Montbeliarde is a common breed in France and is used world-wide for cross-breeding with Holstein. Genetic parameters increase knowledge on the genetic background of claw disorders in Montbeliarde and were estimated in chapter 5.

In the general discussion, chapter 6, possibilities for improved phenotyping or alternative methods to measure claw disorders, were investigated. Accuracies for genomic selection and progeny testing were explored and genetic gain of simulated breeding strategies were discussed.

2

Genetic parameters for claw disorders and the effect of preselecting cows for trimming

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Abstract

Claw disorders are important traits relevant to dairy cattle breeding from an economical and welfare point of view. Selection for reduced claw disorders can be based on hoof trimmer records. Typically, not all cows in a herd are trimmed. Our objectives were to estimate heritabilities and genetic correlations for claw disorders and investigate the effect of selecting cows for trimming. The data set contained 50,238 cows, of which 20,474 cows had at least one claw trimming record, with a total of 29,994 records. Six claw trimmers scored 14 different claw disorders: abscess (AB), corkscrew claw (CC), (inter-)digital dermatitis or heel erosion (DER), double sole (DS), hardship groove (HG), interdigital hyperplasia (IH), interdigital phlegmon (IP), sand crack (SC), super-foul (SF), sole hemorrhage (SH), sole injury (SI), sole ulcer (SU), white line separation (WLS), yellow discoloration of the sole (YD), and a combined claw disorder trait. Frequencies of the claw disorders for trimmed cows ranged from 0.1% (CC, YD, HG) to 23.8% (DER). More than half of the cows scored had at least one claw disorder. Heritability on the observed scale ranged from 0.02 (DS, SH) to 0.14 (IH) and on the underlying scale from 0.05 to 0.43 in trimmed cows. Genetic correlations between laminitis-related claw disorders were moderate to high, and the same was found for hygiene-related claw disorders. The effect of selecting cows for trimming was first investigated by including untrimmed cows in the analyses and assuming they were not affected by claw disorders. Heritabilities on the underlying scale showed only minor changes. Second, different subsets of the data were created based on the percentage of trimmed cows in the herd. Heritabilities for IH, DER, and SU tended to decrease when a higher percentage of cows in the herd were trimmed. Finally, a bivariate model with a claw disorder and the trait “trimming status” was used, but heritabilities were similar. Heritability for trimming status was relatively high (0.09). Genetic correlations of trimming status with claw disorders were generally moderate to high. To conclude, the effect of selecting cows for trimming on the heritability for claw disorders is negligible. Selecting herds with a high fraction of cows being trimmed tended to decrease heritability. Trimming status, as such, is a heritable trait and correlated with claw disorders and is therefore an interesting trait to include in the genetic evaluation.

Key words: foot health, dairy cattle, heritability, trimming status

2.1 Introduction

Claw disorders are among the most important health traits in dairy cattle breeding, with prevalences >70% (Manske et al., 2002; Somers et al., 2003; Van der Waaij et al., 2005; Capion et al., 2009). Claw disorders are usually painful and therefore affect cow welfare (Enting et al., 1997; Bruijnis et al., 2012a,b). In addition, clinical and subclinical claw disorders cause considerable economic losses (Enting et al., 1997; Bruijnis et al., 2010, 2012a,b); claw and leg disorders are the third most economically important trait in dairy cattle after mastitis and fertility problems (Enting et al., 1997). The economic loss due to claw disorders is estimated to be \$75 per cow per year (Bruijnis et al., 2010).

Claw disorders can be divided into different categories according to their etiology: hygiene-related claw disorders [e.g., (inter-)digital dermatitis, interdigital phlegmon, heel horn erosion, and interdigital hyperplasia], nutrition- or laminitis-related claw disorders (e.g., sole hemorrhage, sole ulcer, double sole, and white line problems), and traumatic disorders (e.g., excessive abrasion of the sole and injuries).

Several risk factors for claw disorders have been reported; for example, hygiene, grazing time, herd size, nutrition, parity, and stage of lactation (Toussaint-Raven et al., 1985; Faye and Lescourret, 1989; Enevoldsen et al., 1991a,b; Philipot et al., 1994; König et al., 2005; Sogstad et al., 2005, 2007; Somers et al., 2005a,b; Barker et al., 2009; Chapinal et al., 2010; Kujala et al., 2010). Holstein cows were at higher risk of developing claw disorders compared with Meuse Rhine Yssel (Holzhauer et al., 2006), Norwegian cattle (Baird et al., 2009), and Ayrshire cows (Kujala et al., 2009). These breed differences indicate a genetic background for claw health. Genetic selection provides a means to improve claw health but requires genetic variation. Heritabilities of multiple claw disorders scored by claw trimmers have been estimated previously and range from 0.01 to 0.14 (Huang and Shanks, 1995; Van der Waaij et al., 2005; Van der Linde et al., 2010).

In general, data on claw disorders are collected by hoof trimmers during regular farm visits. Typically, the farmer selects the cows to be trimmed. Having observations on a selected sample might affect the estimated heritabilities. By selecting herds with a high percentage of cows trimmed, the preselection of cows to be trimmed can be reduced because most cows are trimmed; for example, Van der Waaij et al. (2005) only included herds with at least 70% of the cows trimmed, and Van der Linde et al. (2010) only included herds with at least 50% of the cows trimmed. However, selection of specific herds might also have consequences for the estimated genetic parameters. The objective of this research is to estimate the

heritability for different claw disorders of Holstein dairy cows and to investigate the effect of untrimmed cows on the heritability estimates. In addition, genetic and phenotypic correlations among claw disorders will be estimated.

2.2 Materials and Methods

Data

The initial data set contained 56,612 Holstein-Friesian cows. Cows with both parents unknown or with 2 different trimming records on the same date were removed. The final data set contained 50,238 cows, of which 20,474 cows had a claw trimming record with a total of 29,994 claw trimming records. The total number of animals in the pedigree was 212,536. The 50,238 cows descended from 3,603 sires with an average of 13 daughters per sire. The trimmed cows descended from 1,746 sires with an average of 17 daughters per sire, and 333 sires had at least 10 daughters with trimming records. Data were collected by 6 professional claw trimmers, from January 2007 through February 2012 during routine visits to 574 dairy farms in France. Repeated trimming occurred both within and across lactations; 69% of the cows had 1 trimming record, 20% had 2 trimming records, and 11% had 3 or more trimming records. Claw disorders were recorded for the hind legs and scored as a binary trait: 0 = no claw disorder, 1 = claw disorder in at least one hind leg. Table 2.1 gives an overview of the claw disorders that were scored and their abbreviations and definitions. Recorded claw disorders were abscess (**AB**), corkscrew claw (**CC**), digital dermatitis (**DD**), double sole (**DS**), heel horn erosion (**HE**), hardship groove (**HG**), interdigital dermatitis (**ID**), interdigital hyperplasia (**IH**), interdigital phlegmon (**IP**), sand crack (**SC**), super-foul (**SF**), sole hemorrhage (**SH**), sole injury (**SI**), sole ulcer (**SU**), white line separation (**WLS**), and yellow discoloration of the sole (**YD**). We combined DD, ID and HE into one disorder, called dermatitis-erosion (**DER**), because of inconsistency in recording individual claw disorders between trimmers. A combined claw disorder trait that included all disorders was created indicating the absence (score 0) or presence (score 1) of at least one claw disorder.

The farmer decided which cows were to be trimmed. As a result, not all cows present in a herd were trimmed during a routine visit of the claw trimmers. In addition, information was available on cows present in a herd at the moment of trimming (e.g., parity, stage of lactation, and pedigree).

2 Genetic parameters for claw disorders

Table 2.1 Description of the different claw disorders, their abbreviations and frequency.

Claw disorder (abbr.)	Description of claw disorder	Frequency (%)
Dermatitis-erosion (DER)	Combination of DD, ID and HE	23.8
Digital dermatitis (DD)	Skin infection above the heel bulbs or at coronary band, red or black growth or wart-like structures.	-
Interdigital dermatitis (ID)	Inflammation of the skin between the claws.	-
Heel horn erosion (HE)	Heel bulb horn is degrading, the claw is unstable and the erosion forms a dark V-shaped groove.	-
White line separation (WLS)	Separation between the sole and the sole wall.	17.8
Sole hemorrhage (SH)	Bruising of the sole; red or blue coloration.	13.9
Double sole (DS)	Presence of a superficial sole which is separated with a space from the underlying sole.	11.3
Interdigital hyperplasia (IH)	Growth of tissue and / or skin between the claws. Secondary infection is likely to occur.	8.7
Sole ulcer (SU)	Horn is no longer produced, exposing the corium, leading to infection of the corium. In most severe case the tissue protrudes the opening in the sole.	8.7
Sandcrack (SC)	Vertical crack in the claw.	2.3
Abscess (AB)	Infection in the sole caused by external injury.	1.1
Super foul (SF)	Formation of a deep fissure in the skin. When infection becomes chronic, large masses of granulated dermal tissue protrude from the fissure.	0.5
Interdigital phlegmon (IP)	Swelling of the entire foot, with a characteristic odor.	0.4
Sole injury (SI)	Object e.g. a nail or stone, penetrating the sole horn.	0.3
Corkscrew claw (CC)	Rotation of the claw in an inward and upward direction.	0.1
Yellow discoloration (YD)	Yellow discoloration of the sole.	0.1
Hardship groove (HG)	Fissure or crack of the claw wall parallel to the hair-line.	0.1
Combined claw disorder	All claw disorders combined into one score with presence of at least one claw disorder = 1 and absence of claw disorders = 0	54.8

Total number of cows: 20,474

Statistical analysis

The following linear animal model was used:

$$Y_{ijklmn} = \mu + H_i + YS_j + P_k + L_l + Animal_m + PE_n + e_{ijklmno}$$

where Y_{ijklmn} is a claw disorder; μ is the overall mean; H_i is the fixed effect of herd i ; YS_j is the fixed effect of year-season of trimming j (season is defined as spring = March – May, summer = June – August, autumn = September – November, winter = December – February); P_k is the fixed effect of the k^{th} parity ($k = 1, 2, 3$, and ≥ 4); L_l is the fixed effect of the l^{th} lactation stage at trimming ($l = 1$ to 10, group 1-9 are 50 days each, with the first group from 1-50 days, the second group from 50-100 days, etc. Cows with lactation stage ≥ 450 days were combined in group 10); $Animal_m$ is the random additive genetic effect of the m^{th} cow $\sim N(0, \mathbf{A}\sigma_a^2)$, where \mathbf{A} is the additive genetic relationships matrix among cows; PE_n is the random permanent environment effect $\sim N(0, \mathbf{I}\sigma_{pe}^2)$; and $e_{ijklmno}$ is the random residual effect $\sim N(0, \mathbf{I}\sigma_e^2)$, where \mathbf{I} is the identity matrix. Heritabilities and repeatabilities were estimated based on univariate analyses. Heritability was calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

and repeatability as:

$$r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

where σ_a^2 = the additive genetic variance, σ_{pe}^2 = the permanent environmental variance, and σ_e^2 = the residual variance. To quantify the importance of differences between herds on claw disorders, additional analyses were performed using a model where herd was included as a random effect. Genetic and phenotypic correlations between different claw disorders were estimated using bivariate analyses and the same model. Analyses were performed using ASReml v3.0 (Gilmour et al., 2009).

Heritabilities on the Underlying Scale

Claw disorders were scored as 0/1 traits. Underlying the observed binary claw disorders might be a continuous liability to develop claw disorders. The method of

Dempster and Lerner (1950) was used to transform heritabilities to the underlying scale (h_u^2):

$$h_u^2 = \frac{h_o^2 \times z^2}{p \times (1 - p)},$$

where h_o^2 is the heritability on the observed scale, z is the ordinate height of the normal distribution at the threshold point corresponding to p , and p is the frequency of the claw disorder. The standard errors for the heritabilities on the underlying scale were calculated using the same formula.

Selection of Cows for Claw Trimming

When analyzing data on claw disorders, often only a selected sample of the population -the trimmed cows- have observations. To investigate the effect of this selection on heritability estimates, 3 alternative ways of analyzing the data were performed: First, untrimmed cows were included in the analysis, and it was assumed that for untrimmed cows, claw disorders were absent (assigned a score of 0). Second, analyses were based on subsets of the data that were created based on the percentage of cows trimmed per herd and per year. Finally, bivariate analyses were performed in which each claw disorder was analyzed with the trait “trimming status.”

Assuming Absence of Claw Disorders for Untrimmed Cows. In this approach, we assumed that farmers decided not to trim cows with no obvious claw disorders. Therefore, all untrimmed cows were assigned a score of 0 for each claw disorder. Untrimmed cows were included in the analyses on all herd trimming dates they were present in the herd.

Percentage of Trimmed Cows Within a Herd. By selecting herds with a high percentage of cows trimmed, the effect of selection of trimmed cows could potentially be reduced. This was investigated by creating subsets of the data based on the percentage of cows trimmed in the herd. The 3 subsets were 69, and $\geq 70\%$ of the cows being trimmed in the herd. Heritabilities for claw disorders were estimated for each of the subsets. Only trimmed cows were included in the analysis.

Bivariate Analysis of Claw Disorders and Trimming Status. Selection related to trimming can be accounted for by performing a bivariate analysis

including trimming status (Ouweltjes et al., 1988). Therefore, we added the trait “trimming status” to the data. Cows that were trimmed had a score for “trimming status” of 1 (and for each claw disorder a score of either 0 or 1). Cows that were not trimmed had a score for “trimming status” of 0 and a missing record for the claw disorders. Bivariate analyses were performed for “trimming status” with each claw disorder. In addition, univariate analyses were performed to estimate the heritability for “trimming status.”

2.3 Results

Frequency of Claw Disorders for Trimmed Cows The frequency of affected cows varied from 0.1 to 23.8% (Table 2.1), and 54.8% of the trimmed cows had at least one of the claw disorders in one or both hind legs. The data used to calculate the frequency of claw disorders only included the trimmed cows. For untrimmed cows it is uncertain whether they had a claw disorder. The frequency of claw disorders was calculated as the number of trimmed cows with a specific claw disorder between 2007 and 2012 as a fraction of the total number of trimmed cows. The most common disorders were DER, WLS, SH, and DS. Only few cows were affected with SF, IP, SI, CC, YD, or HG.

Systematic Environmental Effects

For most claw disorders, herd explained 1 to 8% of the total variance. However, for some claw disorders, variation explained by herd was substantially higher: 15% for AB, 18% for DER, and 25% for WLS. The year-season effect was significant for all claw disorders ($P < 0.005$). The estimates for the fixed-effect classes indicated that WLS, DS, and IH occurred more often in autumn, SH occurred more in autumn and winter, and DER occurred more in winter and spring. Parity at claw trimming was significant for AB, SH, DS, DER, IH, WLS, SU, and the combined claw score ($P < 0.001$). Parity was not significant for PA, PI, SC, or HG. The estimated parity effects indicate that the frequency of AB, DS, WLS, IH, SC, and HG increased with increasing parities. For DER, frequency decreased with increasing parities. Lactation stage at claw trimming was significant for AB, SH, DS, DER, IH, WLS, SU, PA, HG, and the combined claw score ($P < 0.01$). Lactation stage at claw trimming was not significant for PI or SC. The estimated effect for lactation stage indicated that the frequency of SH was higher from 50 to 200 d in lactation, for SU from 100 to 200 d in lactation, and for DER and WLS from 50 to 100 d in lactation.

2 Genetic parameters for claw disorders

Table 2.2. Heritabilities and repeatabilities for different claw disorders for trimmed cows on the observed and underlying scale. Heritabilities for both trimmed and untrimmed cows, and for different percentages of cows trimmed per herd per year on the underlying scale are included.

Claw disorder	Trimmed		Trimmed & untrimmed		Percentage trimmed cows per herd per yr		
	σ_p^2	r (SE)	h_o^2 (SE)	h_u^2 (SE)	h_{u+}^2 (SE)	h_u^2 (SE)	h_u^2 (SE)
Interdigital hyperplasia (IH)	0.06	0.33 (0.01)	0.14 (0.01)	0.43 (0.01)	0.39 (0.01)	0.46 (0.09)	0.20 (0.06)
Dermatitis – erosion (DER)	0.12	0.04 (0.01)	0.04 (0.01)	0.07 (0.01)	0.07 (0.01)	0.12 (0.04)	0.04 (0.02)
White line separation (WLS)	0.10	0.13 (0.01)	0.04 (0.01)	0.09 (0.01)	0.07 (0.01)	0.10 (0.03)	0.11 (0.03)
Sole ulcer (SU)	0.06	0.12 (0.01)	0.03 (0.01)	0.11 (0.01)	0.08 (0.01)	0.26 (0.07)	0.12 (0.04)
Sole hemorrhage (SH)	0.08	0.02 (0.01)	0.02 (0.01)	0.05 (0.01)	0.04 (0.01)	0.04 (0.03)	0.04 (0.02)
Double sole (DS)	0.07	0.06 (0.01)	0.02 (0.01)	0.06 (0.01)	0.06 (0.01)	0.06 (0.03)	0.08 (0.03)
Combined claw score	0.19	0.07 (0.01)	0.05 (0.01)	0.08 (0.01)	0.08 (0.01)	0.12 (0.04)	0.06 (0.02)

σ_p^2 = phenotypic variance, r = repeatability for claw disorder scores of trimmed cows, h_o^2 = heritability on the observed scale for trimmed cows, h_u^2 = heritability on the underlying scale (Dempster and Lerner, 1950) for trimmed cows, and h_{u+}^2 = heritability on the underlying scale for trimmed and untrimmed cows (assuming absence of claw disorders for untrimmed cows).

2 Genetic parameters for claw disorders

Table 2.3. Frequencies for different claw disorders for all herds and for different percentages (<35%, 35% - 70%, and ≥70%) of cows trimmed per herd per year. The number of cows, herds and average herd size in each subset is indicated.

		Percentage of cows trimmed per herd per year							
		<35%	35% - 70%		≥70%				
All herds		20.474	6.433	13.318	8.556				
Number of trimmed cows		29.764	24.353	5.575	501				
Number of herds		574	386	207	145				
Average herd size		88	80	91	62				
Claw disorder	Group 1*	Group 2**	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	
	Interdigital hyperplasia (IH)	8.7	3.6	11.0	2.3	4.8	3.4	6.2	5.9
	Dermatitis – erosion (DER)	23.8	9.7	22.9	4.8	13.3	9.4	19.4	18.3
	White line separation (WLS)	17.8	7.3	26.1	5.5	8.9	6.3	10.3	9.7
	Sole ulcer (SU)	8.7	3.6	11.2	2.3	4.0	2.8	5.9	5.6
	Sole hemorrhage (SH)	13.9	5.7	11.4	2.4	8.2	5.8	11.2	10.6
	Double sole (DS)	11.3	4.6	13.5	2.8	5.5	3.9	8.7	8.2
	Combined claw score	54.8	22.3	66.7	13.9	30.7	21.6	40.2	37.8

* Group 1 consists of only trimmed cows

**Group 2 consists of trimmed and untrimmed cows, assuming absence of claw disorders for untrimmed cows

Heritabilities and Repeatabilities for Claw Disorders

Table 2.2 shows the heritabilities for claw disorders based on data obtained on trimmed cows, on both the observed and the underlying scales. Estimates are only reported for claw disorders with a frequency of >5%. For other claw disorders, heritability estimates did not differ significantly from zero. When only trimmed cows were included in the analyses, heritabilities on the observed scale were generally low, ranging from 0.02 (SH, DS) to 0.04 (DER, WLS). A moderately low heritability of 0.14 was found for IH. When all claw disorders were combined into one trait, the heritability of having at least one claw disorder was 0.05 (± 0.01). Repeatabilities were generally low (Table 2.2), ranging from 0.02 to 0.13 (± 0.01). The highest repeatability was found for IH (0.33), indicating that once a cow is affected by this claw disorder, it is likely to be affected at subsequent occasions. Heritabilities on the underlying scale were higher, ranging from 0.05 (± 0.01) for SH to 0.43 (± 0.05) for IH. The combined claw disorder score had a heritability of 0.08 (± 0.01) on the underlying scale. Heritabilities and standard errors on the underlying scale, estimated using a threshold model (results not shown), were similar to the estimates obtained using the Dempster and Lerner (1950) transformation.

Selection of Cows for Claw Trimming

Assuming Absence of Claw Disorders for Untrimmed Cows. Heritabilities were also estimated including untrimmed cows (which were assigned a score of 0 for all claw disorders). Frequencies of the claw disorders decreased when untrimmed cows with a score of 0 were added, which would affect heritability estimates on the observed scale. To correct for the difference in frequency, heritability estimates were transformed to the underlying scale (Table 2.2). Heritability estimates were similar compared with the estimates obtained from only including trimmed cows. On the observed scale, heritabilities were lower when untrimmed cows were included with a score of 0 for claw disorders (results not shown), because of the change in claw disorder frequency.

Percentage of Trimmed Cows Within a Herd. Heritabilities on the underlying scale for the 3 subsets based on percentage of cows in the herd trimmed (<35%, 35%-69%, and $\geq 70\%$) are shown in Table 2.2. Only trimmed cows were included in the analyses. In general, heritabilities on the underlying scale showed remarkable differences for some claw disorders across the different subsets, while other claw disorders showed similar results. The heritability for IH, DER and SU showed a tendency to decrease when a higher percentage of cows in the herd was trimmed, although standard errors were high. Heritabilities for WLS and SH were similar

across the different subsets. The frequencies of claw disorders in the three subsets are shown in Table 2.3. In most herds, fewer than 35% of the cows were trimmed. Most trimmed cows were kept in herds where 35% - 69% of the cows were trimmed. Smaller herds seemed to have trimmed a larger percentage of cows in the herd. When only considering trimmed cows (group 1 in Table 2.3), the frequencies of all claw disorders were highest when fewer than 35% of the cows in the herd were trimmed and lowest when 35 to 69% were trimmed. When untrimmed cows (assumed without claw disorders) were included (group 2 in Table 2.3), the frequencies for all claw disorders tended to increase when a higher percentage of cows in the herd was trimmed.

Bivariate Analysis of Claw Disorders and Trimming Status. When performing bivariate analyses in which each claw disorder was analyzed simultaneously with the trait “trimming status,” heritability estimates for claw disorders on the observed scale ranged from 0.02 (DS and SH) to 0.14 (IH) (Table 2.4). Heritabilities and standard errors for all claw disorders on the observed and underlying scale were the same as results from the univariate model given in Table 2.2. Heritability on the observed scale for the trait “trimming status” was 0.09 (± 0.01) and repeatability was also 0.09 (± 0.01). The herd variance was calculated with herd as a random term in the model and explained 56% of the total variance in trimming status. The heritability for trimming status transformed to the underlying scale was 0.14 (± 0.01). The genetic correlations between trimming status and the laminitis-related claw disorders WLS, DS, and SU ranged from 0.20 to 0.65, and with SH it was -0.17 (Table 2.4). The genetic correlation between trimming status and the hygiene-related claw disorder DER was 0.23 and with IH 0.34 (Table 2.4).

Table 2.4. Heritabilities (h_o^2) and genetic correlations (r) from bivariate analyses between trimming status and claw disorders in Holstein dairy.

Claw disorder	r (SE)	h_o^2 (SE)
Trimming status	1	0.09 (0.01)
Interdigital hyperplasia (IH)	0.34 (0.07)	0.14 (0.01)
Dermatitis – erosion (DER)	0.23 (0.07)	0.04 (0.01)
White line separation (WLS)	0.20 (0.10)	0.04 (0.01)
Sole ulcer (SU)	0.65 (0.10)	0.03 (0.01)
Double sole (DS)	0.49 (0.12)	0.02 (0.01)
Sole hemorrhage (SH)	-0.17 (0.10)	0.02 (0.01)
Combined claw score	0.09 (0.09)	0.05 (0.01)

Genetic and Phenotypic Correlations Between Claw Disorders

Table 2.5 contains phenotypic and genetic correlations between claw disorders. Phenotypic correlations were generally weak, ranging from -0.02 to 0.11 . Genetic correlations varied widely with a strong genetic correlation of $0.90 (\pm 0.10)$ between SH and SU. Moderately strong genetic correlations were found between IH and DER (0.66 ± 0.08) and between DS and SH (0.73 ± 0.15). A negative genetic correlation was found between SH and IH (-0.40 ± 0.13).

Table 2.5. Genetic (above diagonal) and phenotypic (below diagonal) correlations between claw disorders in Holstein dairy.

Claw disorder	Interdigital hyperplasia	Dermatitis – erosion	White line disease	Double sole	Sole hemorrhage	Sole ulcer
IH		0.66 (0.08)	0.22 (0.11)	0.28 (0.14)	-0.40 (0.13)	0.04 (0.13)
DER	0.11 (0.01)		0.07 (0.14)	0.07 (0.17)	-0.15 (0.14)	0.07 (0.16)
WLS	0.00 (0.01)	-0.01 (0.01)		0.47 (0.15)	0.10 (0.17)	0.49 (0.13)
DS	0.03 (0.01)	0.00 (0.01)	0.12 (0.01)		0.73 (0.15)	0.04 (0.21) ¹
SH	-0.02 (0.01)	-0.00 (0.01)	0.04 (0.01)	0.03 (0.01)		0.90 (0.10)
SU	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	0.16 (0.01) ¹	0.03 (0.01)	

¹LogL did not converge

2.4 Discussion

Frequency of Claw Disorders

This study includes frequencies of 14 different claw disorders and a combined score for all claw disorders. Highest frequencies were found for DER, SH, and WLS, which is in agreement with previous studies (Manske et al., 2002; Van der Waaij et al., 2005; Holzhauer et al., 2008; Bicalho et al., 2009; Van der Linde et al., 2010). Not all cows in the herds were trimmed, possibly because they were not affected by claw disorders. If only cows with problems were trimmed and all other cows did not have any claw disorders, frequencies of claw disorders based on trimmed cows only would be biased. If we assume that claw disorders were absent for untrimmed cows, 22% of all (trimmed and untrimmed) cows had at least one claw disorder. When only considering the trimmed cows, 55% of them had at least one claw disorder, which is in the range reported in previous studies. Some studies have reported frequencies exceeding 70%, mainly attributable to higher frequencies of DER and SH (Manske et al., 2002; Van der Waaij et al., 2005; Fjeldaas et al., 2006; Van der Linde et al., 2010). However, frequencies lower than 55% have also been

reported (Vaarst et al., 1998; Somers et al., 2003; Fjeldaas et al., 2006; Cramer et al., 2008).

Heritabilities and Repeatabilities of Claw Disorders

Heritabilities for claw disorders were generally low and in line with previous studies (Smit et al., 1986; Huang and Shanks, 1995; König et al., 2005; Van der Waaij et al., 2005; Harder et al., 2006; Van der Linde et al., 2010; Schöpke et al., 2013). A recent study by Oberbauer et al. (2013) found higher heritability estimates, especially for sole ulcer and digital dermatitis (0.30 to 0.40). Heritabilities on the observed scale ranged from 0.02 for DS and SH to 0.14 for IH. Heritabilities for categorical traits are frequency dependent when estimated with a linear model (Gianola, 1982). To correct for differences in claw disorder frequencies, heritabilities on the observed scale can be transformed to the underlying scale using the method proposed by Dempster and Lerner (1950). The repeatabilities for different claw disorders were generally low, indicating that claw scores for a certain cow change over time. When a cow has a claw disorder at a certain trimming occasion, it does not necessarily mean it will have the same claw disorder at a subsequent trimming occasion. This makes repeated observations valuable for estimation of breeding values.

Selection of Cows for Claw Trimming

Assuming Absence of Claw Disorders for Untrimmed Cows. Cows are normally trimmed at the farmer's request. In the current study, about one-third of the cows in a herd were trimmed in a year. Cows might not be trimmed because they do not have any claw disorders, resulting in preselection of cows with phenotypes for claw disorders. In this context, "claw disorders" might not only be claw disorders per se but could also include excessive horn growth. When untrimmed cows were included in the analysis with a claw disorder score of 0, the heritabilities on the underlying scale did not change. This suggests that selection of cows to be trimmed has limited effect on the heritability estimates.

Percentage of Trimmed Cows Within a Herd. To avoid potential bias caused by selecting cows for trimming, some studies selected herds where at least a certain percentage of the cows were trimmed (e.g., Van der Waaij et al., 2005; Van der Linde et al., 2010). However, this could lead to bias due to selecting herds with a specific management. Heritabilities on the underlying scale tended to decrease gradually for IH, DER, SU, and the combined claw score when a higher percentage of cows in the herd was trimmed. As transformation to the underlying scale removes the relationship between frequency and heritability, this effect cannot be

explained by a “frequency effect.” The observed effect could be due to the selection of a specific type of herd management. With improved management, the number of cows displaying claw disorders will be (temporarily) reduced. Cows with a given susceptibility will not develop claw disorders in a herd with good management, whereas they might develop claw disorders under less optimal management. Cows might not have been challenged to express their genotype in optimal environments (Bishop and Woolliams, 2010). In herds without affected animals for a certain claw disorder (i.e., a frequency of 0%), it is not possible to estimate heritabilities. Removing herds without affected animals for a certain claw disorder resulted in a considerable reduction of the data due to low frequencies of claw disorders. This complicates the estimation of heritabilities, although they seemed to increase (results not shown). Bishop and Woolliams (2010) also indicated that heritability estimates were lower when not all animals were triggered to develop the disease. The frequencies of trimmed cows with specific claw disorders also change when the percentage of trimmed cows in the herd changes. These differences could be due to frequencies being expressed relative to the number of cows trimmed. Therefore, untrimmed cows, assumed without claw disorders, were included to calculate the frequencies of claw disorders again. For trimmed cows, the frequency of claw disorders was highest when a low percentage of cows in the herd was trimmed. However, this was likely due to the high number of untrimmed cows. For trimmed and untrimmed cows combined, the frequency of claw disorders was lowest when a low percentage of cows in the herd was trimmed and tended to increase when a higher percentage of cows in the herd was trimmed. Herds with a low percentage of cows trimmed may have a strategy of trimming only cows with severe claw disorders. On the other hand, herds with a low percentage of cows trimmed might have had few cows with claw disorders. Both would result in a low number of cows affected. Similarly, herds with a high percentage of cows trimmed might be herds with more claw disorders. However, it could also be that herds with a high percentage of cows trimmed have adopted a preventive trimming practice. When preventive trimming is performed, cows are usually trimmed on a regular basis whether they have problems or not. Results suggest that selecting herds based on the percentage of cows trimmed results in different heritability estimates and is likely the result of selecting a certain type of herd management. Therefore, care should be taken when selecting herds based on the percentage of cows trimmed. To avoid problems associated with selecting herds, it is recommended to trim all cows in the herd.

Bivariate Analysis of Claw Disorders and Trimming Status. Selection related to trimming can be accounted for by performing a bivariate analysis including trimming status. Heritabilities for claw disorders from the bivariate model were similar to those obtained using a univariate model. A similar approach has been used to adjust for preselection of horses participating in competition (Klemetsdal, 1992; Ducro, 2010). By including competition status and using a bivariate analysis, selection bias was reduced (Arnason, 1999; Ducro, 2010; Albertsdóttir et al., 2011). In the current study, a bivariate analysis did not result in different heritability estimates. This suggests that the selection of cows for trimming does not result in a serious bias, perhaps because cows are selected for trimming based on phenotypic observations; that is, claw disorders. Claw disorders have low heritability (ranging from 0.02 to 0.14), and therefore this selection has only a minor effect on the genetic variance. In contrast, in horses, the heritability for the traits under study range from 0.15 to 0.60 (Arnason, 1999; Ducro, 2010; Albertsdóttir et al., 2011) and selection might, therefore, have a severe effect on heritability estimates. Interestingly, trimming status had a relatively high heritability compared with heritabilities for claw disorders. The heritability for trimming status indicates a genetic background for cows to be trimmed. The need for trimming might be a claw disorder but excessive horn growth might also be a reason for a farmer to select a cow for trimming. Cows that need more frequent claw trimming are not favored and therefore trimming status might be an interesting trait to be included in selection. To our knowledge, this is the first study to investigate “trimming status” as a trait. In general, genetic correlations estimated between trimming status and each claw disorder were positive, indicating that cows that are more likely to be trimmed are also more likely to be affected by a claw disorder. Sole hemorrhage is often an early stage of SU and usually does not cause visible problems for the cow. This might explain the low correlation between trimming status and SH.

Genetic and Phenotypic Correlations Between Claw Disorders

Genetic correlations between claw disorders found in the present study were similar to those reported in previous studies from Van der Waaij et al. (2005) and Van der Linde et al. (2010). The very strong genetic correlation between SU and SH indicates that both traits have a similar genetic background. The claw disorders SH, SU, WLS, and DS have been associated with (sub)clinical laminitis (Ossent and Lischer, 1998) and most have moderate to strong genetic correlations in this study, which supports a common etiology. A moderately strong genetic correlation between 2 hygiene-related disorders, IH and DER, was found. Interdigital

hyperplasia usually develops as a result of chronic irritation of the skin due to infection such as (inter) digital dermatitis and environmental factors as slurry and rocky pasture. Once developed, IH will not go away unless surgically removed. This is also reflected in the higher repeatability of IH compared with other claw disorders. Overall, moderate to high genetic correlations were found among laminitis-related disorders and among hygiene-related disorders, whereas correlations between hygiene- and laminitis-related disorders were generally low or negative. These correlations indicate a different genetic background for laminitis-related and hygiene-related disorders, as was also indicated by Buch et al. (2011). Given the fact that selection of trimmed cows has a negligible effect on heritability estimates, we expected that selection would also not affect estimates of genetic correlations. Therefore, genetic correlations were not estimated with untrimmed cows, assumed without claw disorders, included in the analyses.

2.5 Conclusions

Results from this study indicate that claw disorders are heritable. We conclude that having trimming records on a selected sample of cows does not have a severe effect on the estimated heritabilities. Selecting herds where a certain percentage of cows are trimmed seemed to influence heritability estimates for some claw disorders. This indicates that delimiting the preselection bias from selecting trimmed cows results in a preselection bias of selecting specific herds (e.g., herds with a specific type of management). The trait “trimming status” is heritable and is an interesting trait for genetic evaluation of claw health.

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3

Genome-wide association study for claw disorders and trimming status in dairy cattle

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Abstract

Performing a genome-wide association study (GWAS) might add to a better understanding of the development of claw disorders and the need for trimming. Therefore, the aim of the current study was to perform a GWAS on claw disorders and trimming status and to validate the results for claw disorders based on an independent data set. Data consisted of 20,474 cows with phenotypes for claw disorders and 50,238 cows with phenotypes for trimming status. Recorded claw disorders used in the current study were double sole (DS), interdigital hyperplasia (IH), sole hemorrhage (SH), sole ulcer (SU), white line separation (WLS), a combination of infectious claw disorders consisting of (inter-)digital dermatitis and heel erosion, and a combination of laminitis-related claw disorders (DS, SH, SU, and WLS). Of the cows with phenotypes for claw disorders, 1,771 cows were genotyped and these cow data were used for the GWAS on claw disorders. A SNP was considered significant when the false discovery rate ≤ 0.05 and suggestive when the false discovery rate ≤ 0.20 . An independent data set of 185 genotyped bulls having at least 5 daughters with phenotypes (6,824 daughters in total) for claw disorders was used to validate significant and suggestive SNP detected based on the cow data. To analyze the trait “trimming status” (i.e., the need for claw trimming), a data set with 327 genotyped bulls having at least 5 daughters with phenotypes (18,525 daughters in total) was used. Based on the cow data, in total 10 significant and 45 suggestive SNP were detected for claw disorders. The 10 significant SNP were associated with SU, and mainly located on BTA8. The suggestive SNP were associated with DS, IH, SU, and laminitis-related claw disorders. Three of the suggestive SNP were validated in the data set of 185 bulls, and were located on BTA13, BTA14, and BTA17. For infectious claw disorders, SH, and WLS, no significant or suggestive SNP associations were detected. For trimming status, 1 significant and 1 suggestive SNP were detected, both located close to each other on BTA15. Some significant and suggestive SNP were located close to SNP detected in studies on feet and leg conformation traits. Genes with major effects could not be detected and SNP associations were spread across the genome, indicating that many SNP, each explaining a small proportion of the genetic variance, influence claw disorders. Therefore, to reduce the incidence of claw disorders by breeding, genomic selection is a promising approach.

Key words: association study, hoof lesion, Holstein-Friesian

3.1 Introduction

Breeding goals in dairy cattle focus not only on production traits, but increasing emphasis is also on health and durability traits (Miglior et al., 2005). Claw disorders are common in dairy cattle with a prevalence of more than 70% (e.g., Manske et al., 2002; Van der Waaij et al., 2005). Claw disorders are important because of welfare issues (Enting et al., 1997) and economic impact (Bruijnis et al., 2012a,b). A trait currently not considered but of interest is the need for claw trimming. Some cows need more claw trimming than others and van der Spek et al. (2013) showed that the need for trimming, or “trimming status,” is a heritable trait. High scores for trimming status reflect that daughters of a bull need more trimming, which is unfavorable and which is positively correlated with increased occurrence of claw disorders (Van der Spek et al., 2013).

Genetic selection for reduced claw disorders is difficult because the disorders are not routinely recorded. Indicator traits for claw disorders, which may be more accurate and easier to obtain, are lameness (Laursen et al., 2009; Weber et al., 2013) and feet and leg conformation traits (Van der Waaij et al., 2005; Van der Linde et al., 2010). Scores for feet and leg conformation are routinely collected in most breeding schemes. Previous studies have detected QTL for lameness and feet and leg conformation (Ashwell et al., 1998a,b; Schrooten et al., 2000; Boichard et al., 2003; Buitenhuis et al., 2007). However, to the best of our knowledge, no linkage or GWAS have been reported on claw disorders. The bovine genome sequencing and the emergence of high-throughput genotyping technologies have made it possible to perform genome-wide association studies (GWAS, e.g., Tellam et al., 2009). Genome-wide association studies enable the detection of genetic variants associated with a particular trait or disease, using dense genome-wide markers, also known as SNP (Hirschhorn and Daly, 2005; Matukumalli et al., 2009). Performing a GWAS might add to a better understanding of the development of claw disorders and the need for trimming, when the underlying genetic background can be identified. A GWAS is a good method to detect SNP associations, but some of the results can be false positives. False-positive associations occur especially due to population structure (Goddard and Hayes, 2009). Even if population structure is accounted for in the analysis and stringent significance thresholds are used, false-positive results might occur due to the high chance of some unaccounted for data structure in livestock populations given the large number of tested SNP (Hayes, 2013). To eliminate false positives, associations detected in a GWAS study should therefore be validated in an independent population (Chanock et al., 2007; Hayes, 2013). Therefore, the aim of the current study was to perform a GWAS on several

claw disorders in dairy cows and to validate the results for claw disorders based on an independent data set. In addition, a GWAS will be performed on the trait trimming status using daughter yield deviations (DYD) of bulls.

3.2 Materials and Methods

Analyses were performed based on 2 data sets: one with genotyped cows and one with genotyped bulls. The data set with cows was based on genotyped cows, which also have phenotypes for claw disorders. These data will be referred to as the cow data. The data set with bulls was based on genotyped bulls that have daughters with phenotypes for claw disorders. Phenotypes of cows adjusted for systematic environmental effects were used to calculate the DYD for bulls. Phenotypes from genotyped cows used in the cow data were dropped from calculating DYD for bulls. In this case, no overlap exists in phenotypes between the 2 data sets. The DYD data of bulls were used to validate significant or suggestive SNP detected for claw disorders using the cow data and will be referred to as the bull validation data. The trait trimming status was analyzed with the bull data without removing phenotypes of genotyped daughters. The DYD were calculated and used as a phenotype for bulls. These data will be referred to as the trimming status data.

Phenotypic Data on Claw Disorders

After removing records of cows with both parents unknown or with 2 different trimming records on the same date ($n = 6,374$ records), the data set contained 50,238 cows. The cows descended from 3,603 sires with an average of 14 daughters per sire. Phenotypes on claw disorders were collected by 6 professional claw trimmers, from January 2007 through February 2012, during routine visits on 574 dairy farms in France. The farmer decided which cows were to be trimmed, and as a result, not all cows present in a herd were trimmed. Information on parity, stage of lactation, and pedigree was available on all cows present in a herd at the moment of trimming (including the non-trimmed cows). Of the 50,238 cows, 20,474 had one or more claw trimming records and in total 29,994 claw trimming records were available. Trimming records were repeated within and across lactations; 69% of the cows had 1 trimming record, 20% had 2 trimming records, and 11% had 3 or more trimming records. Claw disorders were recorded for the hind legs and scored as a binary trait: 0 = no claw disorder, 1 = claw disorder in at least one hind leg. Recorded claw disorders used in the current study were double sole (DS), interdigital hyperplasia (IH), sole hemorrhage (SH), sole ulcer (SU), white line separation (WLS), and a combination of infectious lesions (DER) consisting of

(inter-)digital dermatitis and heel erosion. The trimming status trait indicates whether a cow was trimmed (score 1) or not trimmed (score 0) during a visit by the claw trimmer on a specific date. The claw disorders and the trait trimming status are explained in more detail by van der Spek et al. (2013). van der Spek et al. (2013) showed moderate to high genetic correlations between laminitis-related claw disorders (DS, SH, SU, and WLS). Therefore, 4 laminitis-related claw disorders were combined by adding up the scores for the individual traits, resulting in a trait LAMIN, with scores ranging from 0 (no claw disorder) to 4 (all 4 claw disorders present). For trimming status, 50,238 cows with phenotypes were available, and for claw disorders 20,474 cows with phenotypes were available.

Genotypic Data

The DNA was extracted from blood or semen samples. Herds with at least 10% of the cows having claw disorders in previous years were identified and all cows on these herds trimmed in the second half of 2012 or first half of 2013 were sampled. Subsequently, all cows with trimming records available were genotyped. Genotypes of bulls were already available and were included when the bull had daughters with trimming records. In total, 1,771 Holstein-Friesian cows and 506 Holstein-Friesian bulls were genotyped with the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA) for 54,609 SNP. All genotyped cows have phenotypic data available as well, and all genotyped bulls have daughters with phenotypic data. The genotyped cows were kept in 87 herds and descended from 434 sires of which 214 had genotypic data available. One bull had a call rate <95% and was eliminated. Genotypes were analyzed using the Illumina GenomeStudio software. Quality control was performed on the genotypic data and a SNP was only included when the following criteria were met: (1) the minor allele frequency (MAF) >2%; (2) the percentage of missing genotypes across all samples <5%; (3) no strong deviation from Hardy-Weinberg equilibrium (χ^2 values < 600). The last criterion was included as a way to filter out poor-quality SNP. Extreme deviations from Hardy-Weinberg equilibrium are expected to be due to poorly called genotypes. Genotypes of bulls with fewer than 5 daughters with records on claw disorders were eliminated; the number of daughters per bull ranged from 5 to 905. A total of 41,761 SNP for 1,771 cows, 327 bulls with phenotypes for trimming status of 6,824 daughters, and 185 bulls with phenotypes for claw disorders of 18,525 daughters were retained and available for analyses.

Association Study for Claw Disorders—Cow Data

The association of an individual SNP with a claw disorder was estimated using the following linear animal model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{pe} + \mathbf{e},$$

where \mathbf{y} is a vector of observations of the trait; \mathbf{b} is a vector of fixed effects, including herd, year-season of trimming (season is defined as spring = March – May, summer = June – August, autumn = September – November, winter = December – February), parity at trimming (consisting of four classes; 1, 2, 3, and ≥ 4), lactation stage at trimming (consisting of 10 classes; class 1 to 9 are 50 days each, with the first class from 1 to 50 d, the second class from 50 to 100 d, etc. Cows with lactation stage ≥ 450 d were assigned to class 10), and SNP (SNP is treated as a class variable with 2 or 3 classes, depending on the number of genotypes); \mathbf{X} is the incidence matrix for the fixed effects; \mathbf{a} is a vector of animal additive genetic effects and is assumed to follow a multivariate normal distribution $\sim N(\mathbf{0}, \mathbf{A}\sigma_a^2)$, where \mathbf{A} is the additive genetic relationships matrix which consisted of 56,867 animals and σ_a^2 is the additive genetic variance; \mathbf{pe} is a vector of permanent environmental effects $\sim N(\mathbf{0}, \mathbf{I}\sigma_{pe}^2)$ and \mathbf{e} is a vector of residual effects $\sim N(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where \mathbf{I} is the identity matrix, σ_{pe}^2 is the permanent environmental variance and σ_e^2 is the residual variance; \mathbf{Z}_1 is the incidence matrix relating observations to animal effects and \mathbf{Z}_2 is the incidence matrix relating observations to permanent environmental effects.

A linear model was used for the binary traits as it is computationally more feasible as compared with a threshold model. Also, Pirinen et al. (2013) showed that for a GWAS, the logistic regression model can be accurately approximated by the linear model. The heritabilities for claw disorders were fixed at the estimates obtained from the variance component analysis as given by Van der Spek et al. (2013). Variance components for LAMIN, combining the 4 laminitis-related claw disorders, were estimated using the model described by Van der Spek et al. (2013) and resulted in a heritability of 0.07 (± 0.01) and a phenotypic variance of 0.34. Analyses were performed using ASReml v3.0 (Gilmour et al., 2009).

The significance threshold for the GWAS was adjusted for multiple testing using the false discovery rate (FDR). The qvalue package in R statistical software (Storey and Tibshirani, 2003) was used to obtain the FDR. A $FDR \leq 0.20$ will be referred to as suggestive and a $FDR \leq 0.05$ as significant in our study.

When a SNP genotype contained less than 5 cows, the records for this genotype were omitted and the association for this SNP was re-evaluated. When the P-value is ≥ 0.05 , the SNP will be omitted from further analysis.

Association Study—Bull Data

The association of an individual SNP with a claw disorder or trimming status was estimated using the following linear animal model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_1\mathbf{a} + \mathbf{e},$$

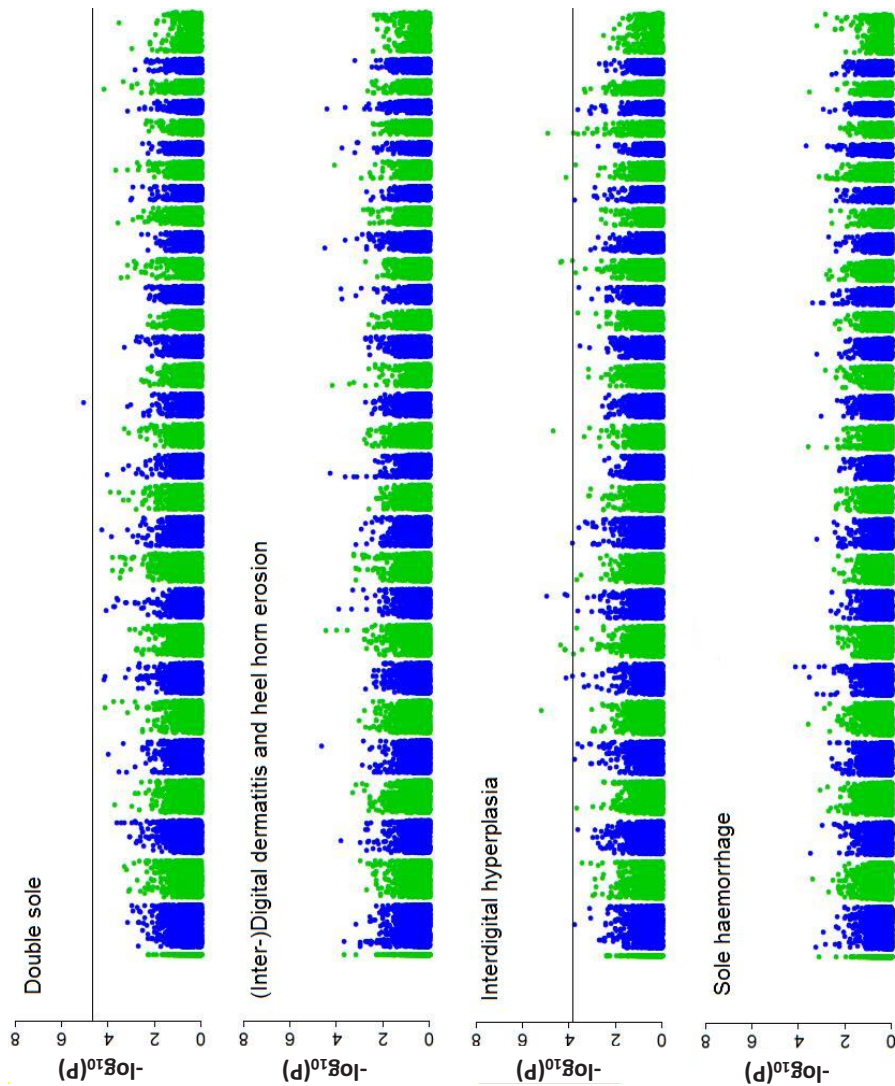
where \mathbf{y} is a vector of observations of the trait; \mathbf{b} is a vector of the fixed effect SNP (SNP is treated as a class variable with 2 or 3 classes, depending on the number of genotypes); \mathbf{X} is the incidence matrix for the fixed effect; \mathbf{a} is a vector of animal additive genetic effects and is assumed to follow a multivariate normal distribution $\sim N(0, \mathbf{A}\sigma_a^2)$, where \mathbf{A} is the additive genetic relationships matrix, which consisted of 2,172 animals, and σ_a^2 is the additive genetic variance; \mathbf{e} is a vector of residual effects $\sim N(0, \mathbf{R}\sigma_e^2)$, where \mathbf{R} is a diagonal matrix with the reciprocal of the reliabilities as diagonal elements and σ_e^2 is the residual variance; \mathbf{Z}_1 is the incidence matrix relating observations to animal effects. Variance components for trimming status and claw disorders were fixed at the estimates obtained from analyses with a weighted univariate linear animal model, with DYC as the dependent variable and animal as a random additive genetic effect, using reliabilities as weights.

Validation of Suggestive SNP for Claw Disorders. In the bull validation set for claw disorders, the effect of a suggestive or significant SNP detected in the cow data was considered validated if it showed a significant effect ($P \leq 0.05$) and if the allele with the favorable effect was identical in both analyses.

Trimming Status. Based on the trimming status data a SNP association was significant if $FDR \leq 0.05$ and suggestive if $FDR \leq 0.20$. When a SNP genotype contained fewer than 5 bulls, the records for this genotype were omitted and the association for this SNP was reevaluated. When the P-value is ≥ 0.05 , the SNP will be omitted from further analysis.

3.3 Results

SNP Analysis on Claw Disorders with the Cow Data



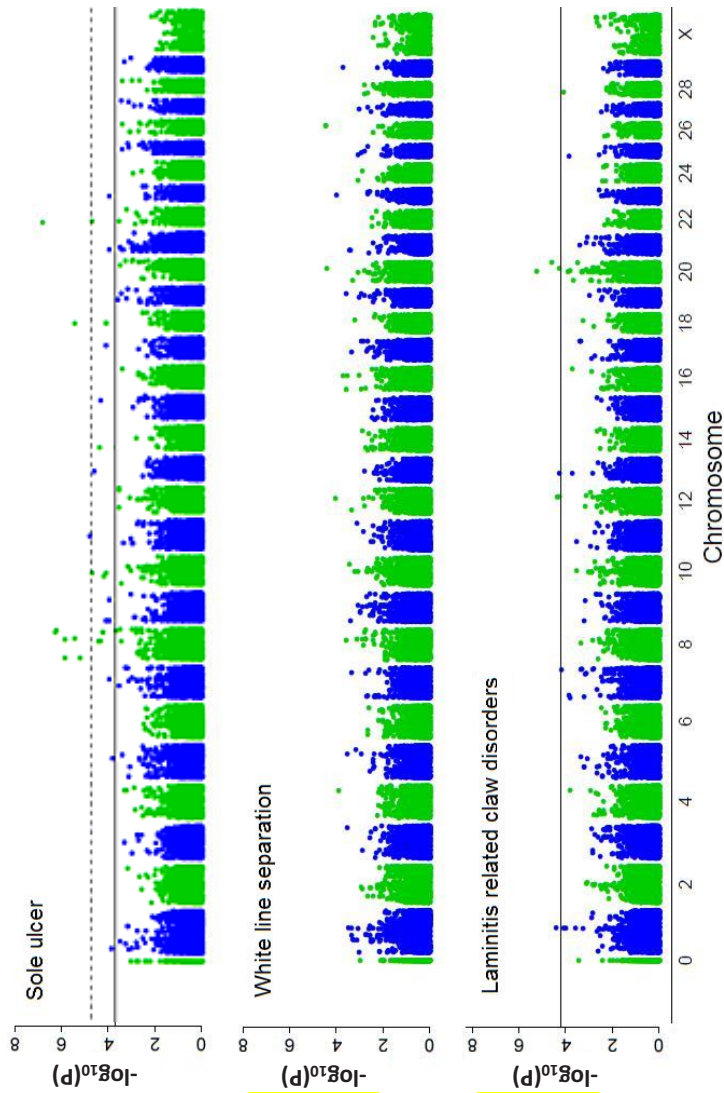


Figure 3.1. GWAS of claw disorders and the combination of laminitis related claw disorders (double sole, sole hemorrhage, sole ulcer, and white line separation). The false discovery rate was set at 0.05 for significant SNP (dashed line) and 0.20 for suggestive SNP (solid line).

The results of the genome-wide association studies for the different claw disorders are shown in Figure 3.1. In total, 17 significant and 77 suggestive SNP were detected. When a genotype contained less than 5 cows, the SNP was reevaluated by omitting the records for this genotype and reevaluating the association for this SNP. Of the 94 significant and suggestive associations, 39 were reevaluated. None of the reevaluated associations had a P-value ≤ 0.05 and were consequently omitted from further analysis; they are also not shown in Figure 3.1. In total, 10 significant and 45 suggestive SNP remained and will be discussed in more detail. Ten significant and 20 suggestive SNP associations were detected for SU. One suggestive association was detected for DS, 17 for IH, and 7 for LAMIN, whereas DER, WLS and SH did not show any suggestive SNP association (Figure 3.1). For 1 significant and 1 suggestive SNP, no records were present for 1 of the 3 genotypes due to low MAF. The chromosome number, position, SNP name, and the number of cows per genotype for the 10 significant SNP with associated claw disorder are shown in Table 3.1 and for the 45 suggestive SNP in Supplementary Table S3.1. The total number of cows per SNP differs slightly due to missing SNP genotypes. The $-\log_{10}$ P-values for the significant SNP associations range from 4.67 to 6.79 (Table 3.1). The most significant SNP ($-\log_{10}$ P-value of 6.79) was associated with an increase in incidence of SU. The AA genotype had an effect size of 0.28 and the BB had an effect size of 0.01, corresponding to an increase in incidence of SU with 27% when a cow has genotype AA as compared with genotype BB. For other significant SNP, the difference between both homozygote genotypes corresponded to an increase in incidence of SU ranging from 10 to 38%. The 10 significant SNP were located on BTA8, BTA10, BTA11, BTA18, and BTA22. Most significant SNP ($n = 5$) were located on BTA8. The 45 suggestive SNP were located on 20 different chromosomes: BTA1, BTA5 to BTA15, BTA17, BTA18, BTA20 to BTA24, and BTA26. Most suggestive SNP were detected on BTA8 ($n = 10$), BTA9 ($n = 5$), and BTA20 ($n = 5$). Estimated SNP effects of the 10 significant SNP are presented in Table 3.1, and estimated SNP effects of the 45 suggestive SNP are presented in Supplementary Table S3.1.

SNP Analyses on DYD of Bulls

Validation of Suggestive SNP for Claw Disorders. Three of the suggestive SNP detected in the GWAS based on cow data were confirmed based on the bull validation data. These SNP were ARS-BFGLNGS-113540 on BTA13 ($P = 0.02$), ARS-BFGLNGS-4929 on BTA14 ($P = 0.02$), and BTB-00678060 on BTA17 ($P = 0.02$). The favorable allele was identical in the cow data as in the bull validation data.

Table 3.1. SNP significantly associated with sole ulcer (false discovery rate (FDR) ≤ 0.05).

BTA	Position (bp)	SNP Name	-log ₁₀ P	FDR	Number of cows			SNP effect		
					AA	AB	BB	AA (SE)	AB (SE)	BB (SE)
8	5437200	BTA-81490-no-rs	5.20	0.02	101	677	992	0.07 (0.03)	0	-0.03 (0.01)
8	5490554	ARS-BFGL-NGS-4062	5.82	0.01	101	668	1002	0.08 (0.03)	0	-0.03 (0.01)
8	74093999	Hapmap43489-BTA-121857	5.85	0.01	1549	209	13	-0.01 (0.02)	0	0.28 (0.06)
8	78533314	ARS-BFGL-NGS-108587	5.44	0.02	6	142	1623	0.38 (0.06)	0	0.001 (0.02)
8	100873051	ARS-BFGL-NGS-34028	6.26	0.01	51	518	1202	0.15 (0.03)	0	-0.02 (0.01)
10	44065746	BTB-01321748	4.67	0.05	1667	104	0	-0.08 (0.02)	0	-
11	48179532	Hapmap38795-BTA-97039	4.79	0.05	1263	464	44	-0.02 (0.01)	0	0.16 (0.02)
18	23878651	ARS-BFGL-NGS-15436	5.42	0.02	1492	264	15	-0.03 (0.01)	0	0.24 (0.04)
22	14300175	BTB-00834995	4.70	0.05	1512	241	13	0.004 (0.01)	0	0.25 (0.04)
22	15276355	ARS-BFGL-NGS-3298	6.79	0.01	15	249	1507	0.28 (0.03)	0	0.01 (0.01)

SNP Associations with Trimming Status. The GWAS results for trimming status are shown in Figure 3.2. Four significant and 6 suggestive SNP associations were detected for the trait trimming status. Eight SNP had one genotype containing 5 or less bulls and therefore these associations were reevaluated by omitting the smallest genotype. None of the re-evaluated associations had a P-value ≤ 0.05 and were consequently omitted from further analysis. Two suggestive SNP associations, both located on BTA15, remained. Figure 3.2 shows the results for trimming status after the reevaluation. The $-\log_{10}$ P-values were 5.13 for SNP UA-IFASA-6898 (32713410 bp) and 4.99 for SNP ARSBFGL-NGS-57210 (32637662 bp).

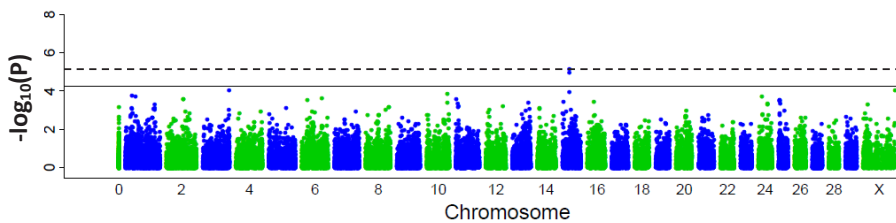


Figure 3.2. GWAS for trimming status using DYD of bulls. The false discovery rate was set at a threshold of 0.05 for significant SNP (dashed line) and 0.20 for suggestive SNP (solid line).

3.4 Discussion

SNP Associations with Claw Disorders in Cows

In the cow data set, 10 significant and 45 suggestive SNP were detected for DS, IH, SU, and LAMIN. The 55 significant and suggestive SNP were located on 20 different chromosomes. This suggests that claw disorders are influenced by many genes dispersed across the entire genome, each explaining a small part of the genetic variance. Caution must be taken because it might be that not all relevant SNP were detected and some SNP might be false positives due to the low number of animals and many SNP suffering from low MAF, as will be discussed later. No suggestive or significant SNP were detected for claw disorders DER, SH, and WLS. Associations with these claw disorders were apparently too small to be detected in the present data set. A larger number of animals will increase the power to detect associations, especially for SNP explaining a small proportion of the genetic variance of the trait (e.g., Visscher, 2008; Goddard and Hayes, 2009). We chose to calculate a trait-based FDR instead of an experiment-based FDR. Adjusting the significance threshold to account for testing multiple traits has some disadvantages because it would penalize studies that report on multiple traits and it would encourage authors to write papers for each trait separately. Claw disorders have a low

heritability (ranging from 0.02 to 0.14, Van der Spek et al., 2013). With 1,771 genotyped cows and assuming a squared correlation (r^2) between marker and QTL of 0.2, allele frequencies of both marker and QTL of 0.5, and a type I error equal to 0.001, the power to detect a QTL can be calculated [using the function `luo.Id.power` (Luo, 1998) from the package `ldDesign` for the statistical software R (Ball, 2010)]. With a proportion of 5% of the phenotypic variance explained by the QTL, the detection power is equal to 74% and with a proportion of 2.5% of the phenotypic variance explained by the QTL, the detection power is equal to 28%. The power calculations show a high probability of identifying SNP explaining at least 5% of the phenotypic variance in our study. Therefore, if a gene with a major effect on claw disorders would be segregating in the current population, it is likely that it would have been detected. The power was too low to detect genes with a moderate or small effect. The GWAS signals detected in the current study are different from what has been reported in other GWAS. The first issue is that in several cases, only a single significant or suggestive SNP was detected in a region, whereas often several SNP in a region show a significant association. This could be due to QTL with a low MAF: 7 of the 10 significant SNP and 23 of the 45 suggestive SNP had one genotype with less than 50 cows and therefore have a low MAF (that alleles with low frequencies can have large effects on complex traits (e.g., Mackay et al., 2012; Weber et al., 2012), the SNP detected in our cow data set are likely overestimated. The significant SNP associations in our study in general have a low MAF and explain a small part of the genetic variance. When the explained genetic variance is low, the detection power is low and effect sizes are likely overestimated (Lynch and Walsh, 1998). This relates to the effect known as the winner's curse (Ioannidis, 2008; Kraft, 2008). The effect sizes for the SNP that are validated based on the bull data are on average almost 4 times smaller as compared with the effect sizes in the cow data. Although SNP effects estimated based on the bull data are allele substitution effects and therefore not fully comparable, it illustrates that the effects reported in Table 3.1 are likely overestimates. The above-mentioned reasons for both issues might explain why only one significantly or suggestively associated SNP with large effect was detected in a region.

Literature

No other genome-wide association studies on claw disorders have been published. However, linkage studies (e.g., Ashwell et al., 2005; Buitenhuis et al., 2007) and a GWAS (Cole et al., 2011) have been published on traits which have been shown to be genetically correlated to claw disorders (Van der Waaij et al., 2005; Laursen et al., 2009; Van der Linde et al., 2010; Weber et al., 2013), for example, rear leg rear

view, rear leg side view, foot angle, general feet and legs score, hock quality, and lameness. First we will discuss how the validated SNP in our study relate to findings in literature and subsequently other significant and suggestive results will be discussed.

The 3 validated SNP were located in chromosomal regions which have been associated with feet and leg conformation traits. The validated SNP ARS-BFGLNGS-113540 (31.3 Mbp) associated with SU in our study is located in the same region of BTA13 as marker UWCA25-BL42 (20–83 cM) associated with foot angle (Ashwell et al., 2005). Another marker detected by Ashwell et al. (2005) associated with foot angle, BMS1899- BM4513 (0–76 cM), was located in the same region of BTA14 as validated SNP ARS-BFGL-NGS-4929 (64.0 Mbp) associated with IH in our study. The third validated SNP, BTB-00678060 (46.3 Mbp) associated with SU in our study, was located in the same region of BTA17 as marker CSSM9-OARFCB48 (32.0 cM) associated with bone quality detected by Buitenhuis et al. (2007).

We searched for candidate genes by taking a 200,000- bp window surrounding the 3 validated SNP. In the region of SNP ARS-BFGL-NGS-113540 on BTA13, the genes *C1QL3* and *PTER* were located. In the region of SNP ARS-BFGL-NGS-4929 on BTA14, the genes *AZIN1*, *TRNAE-UUC*, *KLF10*, *ODF1*, and *UBR5* were located, and in the region of SNP BTB-00678060 on BTA17, the genes *SFSWAP*, *MMP17*, *ULK1*, *PUS1*, *EP400*, *LOC101906627*, and *LOC100138728* were located. However, none of the genes has an apparent function related to claw disorders. Eight suggestive and 3 significant SNP detected based on the cow data set were located close to associations with feet and leg conformation detected in previous research. The SNP ARS-BFGL-NGS-12807 on BTA5 at 71.8 Mbp was suggestively associated with SU in our study. At almost the same location (71.7 Mbp), Cole et al. (2011) detected an association with general feet and leg score. Furthermore, in the same region (Lysmic-ETH10 at 72 cM) Hiendleder et al. (2003) detected a QTL associated with foot angle. Buitenhuis et al. (2007) detected a QTL on BTA8 (marker MCM64-CSSM047 at 92.0 cM) associated with foot angle and this appears to be in a region associated with SU in our study: 3 significant and 5 suggestive SNP in the region 67.8 to 106.6 Mbp. A linkage study by Hiendleder et al. (2003) showed an association of marker MILSTS077 on BTA13 (54 cM) with rear leg side view. This marker appears to be close to SNP ARS-BFGL-NGS-113236 (29.6 Mbp) associated with LAMIN in the current study. On BTA6, Hiendleder et al. (2003) detected marker FBN14 (88 cM), which is associated with foot angle and appears to be close to SNP BTA-77057-no-rs (87.4 Mbp) associated with IH in the current study. Another linkage study detected marker BP7 on BTA6 (85 cM), which is also located

close to SNP BTA-77057-no-rs, and in their study it is associated with rear leg set (Schrooten et al., 2000).

The SNP on BTA18 position 58.7 Mbp detected by Cole et al. (2011) and associated with rear leg side view was also detected in our study. In our study, this SNP was associated with IH, but one genotype had only 3 observations. After removing this genotype, the SNP was no longer significant ($P > 0.05$). A recent study of Swalve et al. (2014) using a preselected set of 384 SNP detected a strong association on BTA21 with SH. The SNP detected by Swalve et al. (2014), SNP rs29017173, had a nominal P-value of 0.06 for SU in our study. The favorable SNP allele in our study is identical to Swalve et al. (2014). For other laminitis-related traits the nominal P-value of this SNP was higher than 0.20. Even though we did not find a significant ($FDR \leq 0.05$) or suggestive SNP ($FDR \leq 0.20$) on BTA21 associated with SH or laminitis-related claw disorders, this result seem to confirm the SNP detected in the study of Swalve et al. (2014).

Whether a cow was trimmed or not, the so-called trimming status of a cow reflects the need for trimming. One significant and one suggestive SNP association was detected, both located close to each other on BTA15. Ashwell et al. (2005) detected a QTL (BMS2684-HBB) located in the same region, associated with stature. The SNP ARS-BFGL-NGS-73559 on BTA26 position 44.7 Mbp, was associated with trimming status but had one genotype with only 2 observations, and when this genotype was removed the SNP was no longer significant ($P > 0.05$). This SNP might still be interesting because it was situated within a region with 7 significant SNP detected by Cole et al. (2011), spanning from 39.3 to 49.2 Mbp. One candidate gene (*SORL1*) for the trimming status trait was detected for both UA-IFASA-6898 and ARSBFGL-NGS-57210 within a 200,000-bp window, but does not have an apparent function related to the trait. Even though we indicated that many significant and suggestive SNP were located in close proximity to SNP identified in previous studies on claw and leg conformation traits, the overlap is not very high. This is most likely due to the rather low to moderate genetic correlations between claw and leg conformation traits and claw disorders, ranging from -0.07 to 0.69 (Van der Waaij et al., 2005; Laursen et al., 2009).

Future Implications

More research with a larger number of animals is needed to obtain a higher power and find more or stronger associations. Claw problems are likely affected by many genes, each explaining a small part of the variation. This complicates identifying causal variants. Further, our results suggest that claw disorders are affected by rare variants with large effects. Identifying causal variants with low MAF might require a

SNP array with higher density (Manolio et al., 2009; Lee et al., 2013). Genomic selection can be used to reduce the incidence of claw disorders, requiring large reference populations.

3.5 Conclusion

The current study is to our knowledge the first GWAS, which revealed significant and suggestive SNP associated with different claw disorders and the need for trimming. In total, 11 significant and 46 suggestive SNP associations were detected for claw disorders and trimming status on 20 chromosomes. Some suggestive SNP were closely located to SNP detected in previous research on feet and leg conformation traits. Another interesting finding was that 3 of the suggestive SNP could be validated in an independent data set of bulls. Also, these 3 SNP were located in the same region on the genome as QTL detected in previous research associated with feet and leg conformation traits. Genes with a major effect were not detected. Likely, many genes each explaining a small proportion of the genetic variance influence the development of claw disorders but the power of the current study was too low to verify this.

3.6 Acknowledgements

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Supplementary Table S3.1. SNP associations for claw disorders and the combination of laminitis related claw disorders (double sole, sole hemorrhage, sole ulcer, and white line separation), that were suggestively (false discovery rate (FDR) ≤ 0.20) associated.

BTA	Position (bp)	SNP Name	claw	disorder*	-log ₁₀ P	Number of cows			SNP effect			
						FDR	AA	AB	BB	AA (SE)	AB	BB (SE)
1	18285474	Hapmap334466-BTA-107178	SU		3.87	0.15	1335	405	28	-0.02 (0.01)	0	0.15 (0.03)
1	93639215	BTA-48243-no-rs	Lam		4.41	0.17	497	898	379	-0.15 (0.03)	0	-0.02 (0.07)
5	71778071	ARS-BFGL-NGS-12807	SU		3.81	0.16	68	520	1183	0.11 (0.03)	0	0.02 (0.01)
6	87365840	BTA-77057-no-rs	IH		5.17	0.06	1204	506	57	-0.01 (0.01)	0	0.16 (0.04)
7	61196630	ARS-BFGL-NGS-5134	IH		4.13	0.12	726	823	222	0.02 (0.01)	0	0.08 (0.02)
7	68156093	Hapmap54765-rs29012520	IH		3.87	0.19	1362	389	20	-0.01 (0.01)	0	0.22 (0.05)
7	68281468	ARS-BFGL-NGS-65419	SU		3.94	0.13	172	822	777	0.08 (0.03)	0	0.03 (0.01)
8	8953354	ARS-BFGL-NGS-69316	IH		3.81	0.20	22	404	1345	0.18 (0.04)	0	0.04 (0.01)
8	24869885	BTB-01052583	IH		4.17	0.12	1500	261	10	0.29 (0.06)	0	-0.03 (0.02)
8	25012621	BTB-01052539	IH		4.17	0.12	10	261	1500	-0.03 (0.02)	0	0.29 (0.06)
8	25035564	ARS-BFGL-NGS-5492	IH		4.17	0.12	10	261	1500	0.29 (0.06)	0	-0.03 (0.02)
8	43241581	ARS-BFGL-NGS-4797	IH		4.37	0.12	564	888	319	0.06 (0.01)	0	0.02 (0.02)
8	67822026	ARS-BFGL-NGS-115977	SU		4.40	0.08	9	204	1558	0.26 (0.05)	0	0.02 (0.02)
8	67846594	ARS-BFGL-NGS-40613	SU		4.34	0.09	9	203	1558	0.26 (0.05)	0	0.02 (0.02)
8	100537840	ARS-BFGL-NGS-79358	SU		4.25	0.10	46	510	1215	0.13 (0.02)	0	-0.01 (0.01)
8	101044054	ARS-BFGL-NGS-112179	SU		4.22	0.10	1013	657	101	-0.02 (0.01)	0	0.07 (0.02)
8	106648392	Hapmap27876-BTA-147931	SU		3.67	0.20	9	217	1545	0.32 (0.06)	0	-0.01 (0.02)
9	1333028	Hapmap41734-BTA-103209	SU		3.94	0.13	1125	578	68	-0.03 (0.01)	0	0.07 (0.04)

3 GWAS of claw health traits in Holstein

9	79256833	Hapmap26214-BTA-163696	SU	3.94	0.13	15	255	1501	0.23 (0.04)	0	0.004 (0.01)
9	83904725	ARS-BFGL-NGS-35130	IH	4.97	0.06	52	494	1225	0.15 (0.05)	0	-0.02 (0.01)
9	85494205	Hapmap59396-rs29023560	IH	4.07	0.13	1244	459	47	-0.02 (0.01)	0	0.15 (0.02)
9	90283190	BTB-00404361	IH	4.18	0.12	1494	270	6	-0.06 (0.02)	0	0.21 (0.12)
10	31162777	BTB-00416921	SU	4.18	0.11	25	399	1347	0.16 (0.03)	0	-0.02 (0.01)
10	44082049	BTB-01413108	SU	4.07	0.11	1662	109	0	-0.09 (0.02)	0	-
11	14134626	Hapmap31534-BTA-26223	IH	3.84	0.19	1027	655	89	0.05 (0.01)	0	0.07 (0.03)
12	62307557	BTA-85739-no-rs	Lam	4.34	0.17	124	721	926	0.10 (0.09)	0	0.14 (0.03)
12	62615139	Hapmap27169-BTA-141662	Lam	4.27	0.17	110	685	976	0.12 (0.09)	0	0.13 (0.03)
13	29647662	ARS-BFGL-NGS-113236	Lam	4.30	0.17	424	908	439	-0.16 (0.08)	0	-0.02 (0.04)
13	31304022	ARS-BFGL-NGS-113540	SU	4.61	0.06	1447	311	13	0.02 (0.01)	0	0.28 (0.05)
14	6203679	Hapmap32486-BTC-056478	SU	4.37	0.08	1136	576	59	0.03 (0.01)	0	0.10 (0.03)
14	63972246	ARS-BFGL-NGS-4929	IH	4.69	0.07	53	497	1221	0.17 (0.05)	0	-0.00 (0.01)
15	52875986	BTB-00600391	DS	5.08	0.07	6	239	1521	0.51 (0.09)	0	0.03 (0.02)
15	67131730	BTB-01649233	SU	4.31	0.09	8	196	1567	0.27 (0.05)	0	-0.02 (0.02)
17	46296022	BTB-00678060	SU	4.09	0.11	6	290	1475	0.44 (0.09)	0	0.01 (0.01)
18	25022927	ARS-BFGL-NGS-24004	SU	4.08	0.11	316	857	598	0.04 (0.02)	0	-0.02 (0.01)
18	25046677	ARS-BFGL-NGS-107737	SU	4.08	0.11	598	856	316	-0.02 (0.01)	0	0.04 (0.02)
20	37721846	BTA-50515-no-rs	Lam	5.25	0.07	970	678	123	-0.01 (0.03)	0	0.27 (0.06)
20	48937082	ARS-BFGL-NGS-59274	Lam	4.27	0.17	878	748	144	-0.02 (0.03)	0	0.22 (0.05)
20	64084187	BTB-00795527	IH	4.38	0.12	1440	312	19	0.03 (0.02)	0	0.23 (0.04)
20	67899804	ARS-BFGL-NGS-6648	IH	4.32	0.12	15	359	1398	0.29 (0.05)	0	0.02 (0.02)

3 GWAS of claw health traits in Holstein

20	71498820	ARS-BFGL-NGS-13702	Lam	4.58	0.16	70	619	1082	-0.05 (0.11)	0	0.14 (0.03)
21	7526820	Hapmap44255-BTA-52888	SU	3.94	0.13	655	889	227	-0.01 (0.01)	0	0.06 (0.02)
23	15725194	ARS-BFGL-NGS-23572	SU	3.94	0.13	6	88	1677	0.36 (0.06)	0	0.03 (0.02)
24	8435459	ARS-BFGL-NGS-107083	IH	4.15	0.12	1513	242	8	0.02 (0.02)	0	0.35 (0.07)
26	9195089	BTA-111275-no-rs	IH	4.91	0.06	780	805	186	-0.01 (0.01)	0	0.09 (0.03)

*DS = double sole, SU = sole ulcer, IH = interdigital hyperplasia, Lam = group of laminitis related claw disorders.

4

Genetic relationships between claw health traits of dairy cows in different parities, lactation stages, and herds with different claw disorder frequencies.

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Abstract

Claw disorders affect cow welfare and profitability of farms. Not only claw disorders but also the need for trimming, “trimming status”, has been shown to be heritable. Limited knowledge is available on whether claw health traits (claw disorders and trimming status) are genetically the same trait in different parities, lactation stages, or in herds with low or high frequency of claw disorders. The aim of the current study was to estimate frequencies, heritabilities and genetic correlations of claw health traits measured in different parities (first vs later parities), in different lactation stages (early lactation vs late lactation), and in herds with different frequencies of claw disorders (low vs high frequency). Analyses revealed that heritabilities measured in different parities, lactation stages or herds with different trait frequencies are similar for most claw health traits. Also, genetic correlations (r_g) for most claw health traits were not found to be different from unity for traits in different parities, lactation stages or herds with different trait frequencies. Sole hemorrhage and infectious lesions were genetically different traits in first or later parities ($r_g = 0.29 \pm 0.31$ and $r_g = 0.66 \pm 0.15$ resp.). White line separation and infectious lesions were genetically different in early and late lactation ($r_g = 0.53 \pm 0.20$ and $r_g = 0.69 \pm 0.13$ resp.) and sole ulcer was genetically different in herds with low or high frequency of sole ulcer ($r_g = 0.75 \pm 0.14$). In our analysis we did not find convincing evidence which supports the use of multiple trait models for the analysis of claw health traits treating them as different traits in different parities, lactation stages, and herds with different claw disorder frequency.

Key words: foot health, Holstein, genetic correlation

4.1 Introduction

The importance of claw health in dairy cattle is well known: claw disorders affect cow welfare and profitability of farms. Studies showed that up to about 70% of the trimmed cows have at least one claw disorder (Manske et al., 2002; Van der Waaij et al., 2005; Van der Spek et al., 2013). Bruijnis et al. (2012) showed that claw disorders have a substantial impact on the cow's welfare. Financial consequences are mainly due to loss in milk production, increased fertility problems, and early culling (Enting et al., 1997; Bruijnis et al., 2010). Average cost in the Netherlands was estimated to be \$95 for a single clinical claw disorder and \$18 for a subclinical claw disorder (Bruijnis et al., 2010).

Many studies have found claw disorders to be heritable (e.g. Gernand et al., 2012; Oberbauer et al., 2013; Van der Spek et al., 2013) and concluded that incidence of claw disorders can be reduced by genetic selection. The need for trimming, "trimming status", is an interesting novel trait for claw health and has been shown to be heritable as well (Van der Spek et al., 2013). For several traits like milk production and fertility, genetic parameters in first parity cows differ from those in later parity cows (Banos and Shook, 1990; Roxström et al., 2001) or genetic parameters differ within parity at different lactation stages (Tijani et al., 1999). Little is known about genetic parameters for claw health traits (claw disorders and trimming status) in first and later parities or early and late lactation. Metabolic factors such as high-concentrate diets (Manson and Leaver, 1987, 1988a, 1988b), restricting forage or dry hay (Livesey and Fleming, 1984; Groehn et al., 1992), calving and the onset of lactation (Webster, 2001; Tarlton et al., 2002) are known to increase the susceptibility to claw disorders. Collard et al. (2000) found an association between high metabolic load and laminitis. As metabolic load has an impact on the development of claw disorders, claw disorders may be genetically different traits in first (when the animal is still growing) or later parities and in early or late lactation. Some studies estimated heritabilities of claw disorders based on first parity records only (Smit et al., 1986; Laursen et al., 2009; Buch et al., 2011). Other studies combined records from multiple parities to estimate heritabilities (e.g. Van der Waaij et al., 2005; Häggman and Juga, 2013; Van der Spek et al., 2013). Harder et al. (2006) found a higher heritability in first parity as compared to first and later parities together, for a combined claw and leg disease trait. Van der Linde et al. (2010) showed that the genetic correlation between first and later parities for digital dermatitis, sole ulcer and interdigital hyperplasia were significantly different from unity. Van der Linde et al. (2010) also showed that heritabilities differed between parities for some claw disorders. Gernand et al.

(2013) showed that genetic correlations were high for the same claw disorders among test-days from mid-lactation (50 to 350 days) but low between early (0-50 days) and late lactation (>305 days).

Bishop and Woolliams (2010) argued that heritabilities for disease resistance are higher under specific environmental conditions where the animal is able to express resistance to disease. Genetic selection might be more effective based on records collected on herds with higher frequencies of claw disorders. In high frequency herds, cows are more likely to express their resistance to claw disorders. Richardson et al. (2014) found a higher heritability for susceptibility to bovine tuberculosis (bTB) for herds with a higher bTB prevalence.

Furthermore, Richardson et al. (2014) found genetic correlations different from unity for susceptibility to bTB between environments that differed in bTB herd prevalence.

Calus et al. (2006) found similar results for somatic cell score across herds with different mean bulk tank somatic cell score. For claw health traits it is unknown whether they are the same traits in herds with different trait frequency.

The aim of the current study was to estimate frequencies, heritabilities and genetic correlations of claw health traits measured in different parities (first vs later parities), in different lactation stages (early lactation vs late lactation), and in herds with different frequencies of claw health traits (low vs high frequency).

4.2 Materials and Methods

Data

The initial dataset contained 67,213 Holstein Friesian cows. Cows with both parents unknown or with two different trimming records on the same date were removed (likely, these records are mistakes as cows are normally not trimmed twice on the same day). Observations with missing parity or lactation stage were removed as well. The final dataset contained 44,317 cows, of which 24,133 cows had at least one claw trimming record, the other cows were present in the herds at time of trimming but were not trimmed themselves. The total number of claw trimming records was 35,966. The farmer decided if and which cows were to be trimmed. As a result, not all cows present in a herd were trimmed during a routine visit of the claw trimmers. At least two cows were trimmed during a herd visit. Data was collected by twelve professional claw trimmers, employed by two claw trimming organizations, from January 2007 until May 2014 during routine visits to 655 dairy farms in France. Claw disorders were recorded for the hind legs and scored as a binary trait: 0 = no claw disorder and 1 = claw disorder in at least one hind leg.

Recorded claw disorders used in the current study were double sole (DS), interdigital hyperplasia (IH), sole hemorrhage (SH), sole ulcer (SU), white line separation (WLS), and a combination of infectious lesions (DER) consisting of (inter-)digital dermatitis and heel horn erosion. The trait “trimming status” indicates whether a cow was trimmed (score 1) or not trimmed (score 0) during a visit by the claw trimmer. The claw disorders and the trait “trimming status” are explained in more detail by Van der Spek et al. (2013).

Statistical Analyses

The frequency of each claw disorder in trimmed cows was calculated as the number of cows with a claw disorder divided by the total number of trimmed cows ($n = 24,133$). Note that not all cows were trimmed. The frequency of “trimming status” indicates how many cows were trimmed of the total number of cows present at the time of trimming.

The following linear animal model was used for univariate and bivariate analyses:

$$Y_{ijklm} = \mu + H_i + YS_j + P_k + L_l + Animal_m + e_{ijklm}$$

where Y_{ijklm} is a claw disorder; μ is the overall mean; H_i is the fixed effect of herd i ($i = 1$ to 655); YS_j is the fixed effect of year-season of trimming j (season is defined as spring = March – May, summer = June – August, autumn = September – November, winter = December – February); P_k is the fixed effect of the k^{th} parity ($k = 1, 2, 3$, and ≥ 4); L_l is the fixed effect of the l^{th} lactation stage at trimming ($l = 1$ to 10, group 1 to 9 are 50 d each, with the first group from 1 to 50 d, the second group from 50 to 100 d, etc. Cows with lactation stage ≥ 450 d were combined in group 10); $Animal_m$ is the random additive genetic effect of the m^{th} cow $\sim N(0, \mathbf{A}\sigma_a^2)$, where \mathbf{A} is the additive genetic relationships matrix among animals; and e_{ijklm} is the random residual effect $\sim N(0, \mathbf{I}\sigma_e^2)$, where \mathbf{I} is the identity matrix. The pedigree consisted of 175,972 animals. Heritability was calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2},$$

where σ_a^2 = the additive genetic variance and σ_e^2 = the residual variance. Analyses were performed using ASReml (Gilmour et al., 2009). Genetic correlations were estimated using bivariate analyses. A genetic correlation significantly different from

one was considered to indicate genetically different traits. The significance of non-unity genetic correlations was tested by constraining the genetic correlation to 0.99 and comparing the likelihood of this model to that of the unconstrained model with a log-likelihood ratio test (LRT). $LRT = -2 * [\log L(H_0) - \log L(H_a)]$, where H_0 indicates the constrained model (i.e. traits are genetically the same) and H_a indicates the unconstrained model and $LRT \sim \chi^2_1$. A genetic correlation was significantly different from one when $P < 0.05$.

First parity versus later parities

To investigate whether a claw disorder (or trimming status) in first and later parity is genetically identical, cows were divided in first and later parity and analyzed using bivariate analysis. Only the first observation of each cow for first and later parities was used.

The number of trimmed cows in first parity was 11,029 and the number of cows in later parities was 16,585. The total number of cows in first parity, including untrimmed cows, was 25,231 and in later parities the total number of cows was 28,765. The average number of cows trimmed per herd was 40 in first parity and 52 in later parities.

Early lactation versus late lactation

To investigate whether a claw disorder (or trimming status) during early or late lactation are genetically identical, cows were divided in groups based on the number of days in lactation at the time of trimming. Early lactation was defined as 0 to 149 days in lactation and late lactation was defined as 150 to 305 days in lactation. Only the first observation in early or late lactation was used. The number of trimmed cows during early lactation was 11,300 and during late lactation 11,139 cows. The average number of cows trimmed per herd was 39 for early lactation and 37 for late lactation. The number of cows with a record for trimming status during early lactation was 24,637 and during late lactation 33,200 cows.

Low versus high frequency

Herds were divided in low (group Low) and high (group High) frequency of a specific claw disorder or trimming status to investigate whether the same trait measured in herds with a low and high frequency are genetically identical. Only the first observation for each cow was used. Low frequency was defined for each trait separately, in such a way that the number of animals in group Low was approximately equal to the number of animals in group High. In general, group Low indicated below average herd frequency of a specific claw disorder and group High

above average. In group Low and High the average number of cows trimmed per herd ranged from 32 to 47 for different claw disorders, with an average of 36 for group Low and 39 for group High.

4.3 Results

First parity versus later parities

Table 4.1 shows the frequencies of claw disorders and trimming status in first parity and later parities. The frequency of IH and WLS increased substantially from first to later parities. The frequency of IH increased from 5.7% to 11.1% and the frequency of WLS increased from 10.8% to 20.5%. The frequency of SH decreased from 28.3% in first parity to 19.3% in later parities. In first parity, 43.6% of the cows were trimmed and in later parities 57.7% of the cows were trimmed.

For most traits, heritabilities were the same in first and later parities (Table 4.1). Heritability for IH increased from first to later parities. IH had a heritability of 0.05 (± 0.01) in first parity which increased to 0.12 (± 0.02) in later parities. Heritability for WLS and DER decreased from 0.06 (± 0.01) in first parity to 0.04 (± 0.01) in later parities. Heritabilities from univariate and bivariate analyses were similar (results not shown). Table 4.1 also shows genetic correlations and the P-value of the LRT, indicating whether the genetic correlation is significantly different from one. The genetic correlation between first and later parities did not significantly differ from one for DS, IH, WLS, SU, and trimming status. A genetic correlation between first and later parities of 0.29 (± 0.31) was estimated for SH and a genetic correlation of 0.66 (± 0.15) for DER. Both correlations were significantly ($P < 0.05$) different from one. For DS a genetic correlation of 0.49 (± 0.25) was found. However, this correlation was not significantly different from one ($P = 0.06$).

Early lactation versus late lactation

Table 4.2 shows the frequencies of claw disorders and trimming status during early and late lactation. Frequencies of claw disorders were generally similar in early and late lactation. The largest differences were found for WLS and SH. WLS increased from 15.2% in early lactation to 18.2% in late lactation. Whereas SH decreased from 28.5% in early lactation to 21.5% in late lactation. For trimming status a large difference was found. During early lactation 45.9% of the cows were trimmed and during late lactation 33.6% of the cows were trimmed. Heritabilities estimated from univariate and bivariate analyses were similar (results not shown). Table 4.2 shows that heritabilities were similar during early and late lactation. Heritabilities for the different disorders ranged from 0.01 to 0.09 and the largest absolute

difference between early and late lactation was 0.02 for IH, SU and WLS. Table 4.2 also shows the genetic correlation and P-value of the LRT for each claw disorder and trimming status. A genetic correlation between early and late lactation of 0.69 (± 0.13) was found for DER and a genetic correlation of 0.53 (± 0.20) was found for WLS. Both were significantly lower than one. The genetic correlation between early and late lactation was not significantly different from one for DS, IH, SH, and SU.

Low versus high frequency

The overall frequency, and the frequency and number of cows in group Low and High of each claw disorder in trimmed cows and of trimming status are shown in Table 4.3. The overall frequency ranged from 8.2% (SU) to 33.9% (DER) for trimmed cows and 54.5% of the cows were trimmed. The cut-off value to divide herds in group Low and High ranged from a herd average of 6% (IH) to 35% (DER). The average frequency of claw disorders in trimmed cows in group Low ranged from 2.4% (IH) to 16.3% (DER). The average frequency of claw disorders in trimmed cows in group High ranged from 13.6% (SU) to 55.2% (DER). On average 24.1% of the cows were trimmed in group Low and 79.7% of the cows in group High.

Heritabilities estimated from univariate and bivariate analyses were similar (results not shown). For most traits heritabilities were similar in group Low and High (Table 4.3). IH and SU had a lower heritability in group Low (both 0.04) as compared to group High (0.14 and 0.08, resp.).

Table 4.3 also shows the genetic correlation and P-value of the LRT in group Low and High. For SU a genetic correlation of 0.75 (± 0.14) between group Low and High was found which was significantly different from one. For all other traits, the genetic correlations between group Low and High was not significantly different from one.

4.4 Discussion

First versus later parities

The frequency of claw disorders in trimmed cows was higher in first parity for some traits (SH, DER, SU) and higher in later parities for other traits (DS, IH, WLS). The frequencies for claw disorders were based on trimmed cows only and therefore might be biased. The frequency of trimming status was higher in later parities.

Table 4.1. Frequencies and heritabilities (estimated with bivariate models) of claw disorders and trimming status in first and later parities. Genetic correlation (r_g) of the same trait in first and later parities and P-values to test whether r_g is lower than one.

Trait and abbreviation	Frequency*		h^{2**}		r_g		P
	parity 1	parity ≥ 2	parity 1	parity ≥ 2	parity 1	parity ≥ 2	
Double sole	DS	9.0%	13.0%	0.02	0.49 \pm 0.25	0.06	0.06
Infectious lesions	DER	37.5%	34.0%	0.06	0.66 \pm 0.15	0.02	0.02
Interdigital hyperplasia	IH	5.7%	11.1%	0.05	0.99 \pm 0.09	1.00	1.00
Sole hemorrhage	SH	28.3%	19.3%	0.01	0.29 \pm 0.31	<0.001	<0.001
Sole ulcer	SU	8.6%	7.6%	0.07	0.96 \pm 0.08	0.73	0.73
White line separation	WLS	10.8%	20.5%	0.06	1.00 \pm 0.08	1.00	1.00
Trimming status		43.6%	57.7%	0.02	0.66 \pm 0.21	0.96	0.96

Table 4.2. Frequencies and heritabilities (estimated with bivariate models) of claw disorders and trimming status during early (0 to 149 DIM) and late (150 to 305 DIM) lactation. Genetic correlation (r_g) of the same trait during early and late lactation and P-values to test whether r_g is lower than one.

Trait and abbreviation	Frequency*		h^{2**}		r_g		P
	early	late	early	late	early	late	
Double sole	DS	11.2%	13.0%	0.01	0.82 \pm 0.21	0.35	0.35
Infectious lesions	DER	37.5%	35.3%	0.04	0.69 \pm 0.13	<0.001	<0.001
Interdigital hyperplasia	IH	8.7%	9.5%	0.07	0.99 \pm 0.08	0.32	0.32
Sole hemorrhage	SH	28.5%	21.5%	0.02	0.94 \pm 0.35	0.89	0.89
Sole ulcer	SU	8.8%	9.8%	0.05	0.99 \pm 0.07	1.00	1.00
White line separation	WLS	15.2%	18.2%	0.04	0.53 \pm 0.20	<0.001	<0.001
Trimming status		45.9%	33.6%	NC***	NC***	-	-

* Claw disorder frequency in trimmed cows only. For trimming status the frequency indicates the proportion of animals trimmed.

** Standard errors for heritabilities were 0.01 or 0.02. *** Not Converged.

4 Genetic analyses of claw health traits

Table 4.3. Frequencies per trait and subdivision in herds with low and high frequencies of a particular claw disorder or trimming status. The number of cows in low and high frequency herds is indicated together with the heritabilities (estimated with bivariate models) of claw disorders and trimming status. Genetic correlation (r_g) of the same trait in low and high frequency herds and P-values to test whether r_g is lower than one.

Trait and abbreviation	Frequency*	Cut-off**	Frequency* (N cows)		h^2 ***		High	r_g	P
			Low	High	Low	High			
Double sole	DS	11.4%	4.9% (12.432)	18.2% (11.701)	0.01	0.01	0.99±0.77	1.00	
Infectious lesions	DER	33.9%	16.3% (12.587)	55.2% (11.546)	0.04	0.04	0.99±0.13	0.89	
Interdigital hyperplasia	IH	8.4%	2.4% (11.826)	14.2% (12.307)	0.04	0.14	0.82±0.12	0.15	
Sole hemorrhage	SH	23.3%	9.9% (12.270)	37.2% (11.863)	0.02	0.01	0.99±0.48	1.00	
Sole ulcer	SU	8.2%	3.2% (12.566)	13.6% (11.567)	0.04	0.08	0.75±0.14	0.03	
White line separation	WLS	17.0%	6.3% (12.537)	28.6% (11.596)	0.03	0.03	0.93±0.18	0.75	
Trimming status		54.5%	24.1% (20.133)	79.7% (24.184)	0.03	0.01	0.70±0.22	0.17	

* Claw disorder frequency in trimmed cows only. For trimming status the frequency indicates the proportion of animals trimmed.

** Standard errors for heritabilities were 0.01 or 0.02

*** Cut-off value; herds with a trait frequency below this value were assigned to the Low frequency group and a frequency equal or higher are assigned to the High frequency group.

When untrimmed cows would be included, and assumed to have no claw disorders, frequencies for all claw disorders were higher in later parities except for SH. A higher frequency or risk for SH in first parity was also found in previous studies (Manske et al., 2002; Sogstad et al., 2005).

Genetic parameters were estimated using a linear animal model for the analysis of binomial traits. It is well documented that heritabilities from these models are frequency dependent. A threshold model accounts for differences in frequencies (Gianola, 1982). However, threshold models are computationally more demanding and resulted in convergence issues as was also explained in Misztal et al. (1989). When the threshold model did converge, it was shown that biased estimates were obtained for herd-year variances and additive genetic effects (Boettcher et al., 1999; Luo et al., 2001). Genetic correlations estimated with linear or threshold models are theoretically expected to be similar (Gianola, 1982;) as was also shown empirically by simulation (Mäntysaari et al., 1991) and using field data (Mao, 1976). Therefore, in the current study a linear animal model was used to calculate genetic parameters.

Heritabilities (Table 4.1) seemed to be different between parities for SH and WLS and was significantly different for IH, but this could be due to a difference in frequency of claw disorders. The frequency of SH was higher in first parity, whereas the frequency of IH and WLS was higher in later parities. When we used a threshold model to estimate heritabilities, on the underlying scale and thereby accounting for a difference in frequency, most heritabilities were similar in first and later parities (results not shown) except for SH. On the underlying scale the heritability for SH in first parity was 0.03 (± 0.02) and in later parities 0.07 (± 0.02).

Genetic correlations were estimated between claw disorders in first and later parities. Convergence of the model could not be reached when claw disorders were analyzed as 3 different traits (parity 1, 2, and ≥ 3). SH and DER were found to be genetically different traits when measured in first or later parities (Table 4.1), suggesting these traits are influenced by different genes in first or later parities. Van der Linde et al. (2010) found digital dermatitis, SU, and IH to be genetically different traits in first and later parities. Digital dermatitis was not included in our analysis as a separate trait but combined with heel erosion and interdigital dermatitis in the trait DER. In our study, DER was also found to be genetically different in first or later parities. We showed that WLS was genetically the same trait in first and later parities, which was also shown by Van der Linde et al. (2010). Cows are undergoing strong physiological changes during the onset of lactation, after first calving. Several traits related to the physiology of the cow have found to be genetically different traits in first and later parities, such as calving ease (Luo et

al., 2002) and gestation length (Eaglen et al., 2012). Lisscher and Ossent (2002) indicated that fat tissue in the digital cushion of the claw develops during first parity. They indicated that heifers have less fat tissue in the digital cushion, which may cause the cushions to be more vulnerable for SH. This might be one reason why SH is genetically different when measured in first or later parities.

In our study we also found infectious lesions (DER) to be genetically different in first and later parities. This finding is in line with findings for other infectious traits such as mastitis or somatic cell score which were shown to be genetically different in first and later parities (Pösö and Mäntysaari, 1996; Banos and Shook, 1998; Carlén et al, 2004).

Early lactation versus late lactation

Heritabilities during early and late lactation were similar, except for IH, SU, and WLS, although the difference was not significant. Gernand et al. (2013) also indicated that heritability estimates for claw disorders were relatively stable from 50 to 305 days in milk. They also found that genetic correlations among different test days were close to 1 from 50 to 305 days in lactation. A low correlation was found between test days from early (0-50 days) or late (>305 days) lactation and mid-lactation (50-305 days). Gernand et al. (2013) argued that this could be due to the low number of observations before day 50 and after day 305 combined with the use of a random regression model. Genetic correlations were estimated between claw disorders in early and late lactation. In our study DER and WLS were genetically different during early or late lactation, indicating an influence of different genes. Convergence of the model could not be reached when claw disorders were analyzed as 3 different traits (early, mid, and late lactation).

Van Knegsel et al. (2007) showed that a negative energy balance during early lactation affects innate immune function and this can influence the development of infectious diseases. Schöpke et al. (2013) found a lower body condition score, indicating a lower energy balance, to be associated with a higher prevalence of digital dermatitis in first parity cows. In our study, DER is a combination of infectious claw disorders including digital dermatitis. Frequency of DER in trimmed cows was slightly higher in early lactation as compared to later lactation, indicating a lower energy balance during early lactation.

During the first part of the lactation, cows experience an increased metabolic load due to recovery from parturition and increasing production combined with a slower increase in feed intake. A high metabolic load often results in a negative energy balance which reaches a peak around 100 days in lactation. When the animal is not able to adapt adequately (e.g. by mobilizing body reserves) to a negative energy

balance, this can result in metabolic disorders such as ketosis (Gillund et al., 2001) and milk fever (Roche and Berry, 2006). Collard et al. (2000) showed that laminitis was unfavorably associated with measures of energy balance. Negative energy balance occurs in early lactation and may be the reason for the genetic correlation of the metabolic disorder WLS to be significantly different from one between early or late lactation. Other metabolic claw disorders (SH, DS, SU) did not show a significant difference during early or late lactation.

Low versus high frequency

Except for SU, claw disorders and trimming status were genetically identical traits when measured in herds with a low or high disorder frequency. For IH and SU the heritability was significantly higher in herds with a high claw disorder frequency. For trimming status the opposite was found, but was not significant. However, when heritabilities were calculated with a threshold model taking the differences in disorder frequency into account, heritability estimates were similar in low or high frequency herds. Based on these results, we conclude that collecting data from herds with different disorder frequency levels does not influence genetic parameters for most traits. Bishop and Woolliams (2010), however, indicated that a higher disease frequency results in a higher heritability due to more animals being exposed to factors triggering the disease. We did not observe that in our study, which could be due to the groups not being extreme enough.

Genetic correlations were estimated between claw disorders in low and high claw disorder frequency herds. Convergence of the model could not be reached when claw disorders were analyzed as 3 different traits (low, medium, high frequency). Next to that, the 'true' claw disorder frequency is not known as not all animals were trimmed. There were less untrimmed cows in low as compared to high frequency herds (24.1% in low vs 79.7% in high frequency herds). The average difference between low and high frequency herds was 20.7%, but with untrimmed cows included, assuming they had no claw disorders, the average difference dropped to 9.8%. Perhaps herds should be divided in low and high frequency herds based on frequencies including untrimmed cows. However, it is unsure whether untrimmed cows were really without claw disorders. Scoring all cows in all herds would overcome this problem by identifying the 'true' frequencies and may lead to higher heritability estimates for high frequency herds. It may also lead to a rearrangement of herds in high or low frequency groups. Even though heritability corrected for frequencies were similar in low or high frequency herds, SU was found to be a genetically different trait. With an average of 3.2% of SU in low frequency herds, this group has a quite extreme frequency of group Low.

Application

Some claw disorders were found to be different traits when measured in different parities or lactation stages, or in herds with low or high claw disorder frequency. The difference of SH in first and later parities was highly significant. For DER and WLS the difference was highly significant between early or late lactation. A multi trait model could be used to estimate genetic parameters and breeding values for traits that are significantly different from unity. In a multi trait model, claw disorders scored in different parities or lactation stages can be treated as separate traits. Another option could be the use of a random regression model for genetic evaluation but many parameters need to be estimated (Jensen, 2001). A single trait model, for example treating claw disorders in first and later parities as the same trait, is easier to implement as compared to a multi trait model. A single trait model may still be preferred if genetic correlations between for example claw disorders measured in first and later parities are high (e.g. above 0.90). In our analysis we did not find convincing evidence which supports the use of multiple trait models for the analysis of claw health traits in different parities, lactation stages, and herd frequency levels.

4.5 Conclusion

The current study shows that most claw disorders and trimming status are genetically the same trait in different parities, lactation stages or herds with low or high claw disorder frequency. DER was found to be a genetically different trait in first and later parities and in early and late lactation. SH was genetically different in first and later parities, WLS was genetically different in early and late lactation, and SU was genetically different in herds with a low or high sole ulcer frequency. The current study is the first to investigate whether trimming status is genetically the same trait in different parities, lactation stages and in herds with a different frequency of trimmed animals. Overall, around 50% of the cows were trimmed and no genetic differences were found due to parity or frequency of trimming in the herd.

4.6 Acknowledgements

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5

Genetic background of claw disorders and trimming status in Montbeliarde cows.

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Concept paper

Abstract

Little is known about claw health in Montbeliarde cows and about the genetic background of the need for trimming. Aim was to estimate frequencies and heritabilities for claw disorders and trimming status in Montbeliarde cows. In addition, genetic correlations between different claw disorders and trimming status were estimated. The final dataset contained 10,766 cows, of which 5,435 cows had at least one claw trimming record with a total of 10,233 trimming records. Data was also available for untrimmed cows present in the herd during claw trimming occasions, but not trimmed themselves. Six claw disorders were scored as binary traits by professional claw trimmers: (inter)digital dermatitis and heel horn erosion (DER), interdigital hyperplasia (IH), double sole (DS), sole hemorrhage (SH), sole ulcer (SU), and white line separation (WLS). The prevalence of claw disorders in trimmed cows ranged from 9.4% (IH) to 41.1% (SH), with 73% of the trimmed cows having at least one claw disorder. Heritabilities for claw disorders ranged from 0.01 (DER) to 0.09 (WLS) on the observed scale, and from 0.02 (DER) to 0.16 (IH, WLS) on the underlying scale. Heritability for trimming status was 0.06 on the observed scale and 0.11 on the underlying scale. Claw disorders and trimming status are heritable, indicating that genetic selection to improve claw health is feasible in Montbeliarde. In addition, the analysis confirmed that trimming status should be considered in breeding programs for improved claw health.

Key words: dairy, foot lesions, genetic background

5.1 Introduction

Impaired claw health can have a major impact on cow welfare (Enting et al., 1997; Bruijnis et al., 2012b). Consequences of impaired claw health include reduced movement and impaired intake of food and water (Metz and Bracke, 2005), which also has negative consequences for milk production and fertility (Melendez et al., 2003; Hernandez et al., 2005). Impaired claw health is the third greatest economic loss in dairy cows, after mastitis and fertility problems (Enting et al., 1997). Claw disorders in Holstein cows costs the farmer on average €53 per cow per year, mainly due to loss of milk production (44%) and increased involuntary culling (22%) (Bruijnis et al., 2010, 2012a). In comparison, cost of mastitis ranges from €65 to €182 per cow per year (Huijps et al., 2008). Claw disorder frequencies in Holsteins range from 50% to even higher than 70% of the trimmed cows having at least one claw disorder (Van der Waaij et al., 2005; Häggman and Juga, 2013; Van der Spek et al., 2013; Weber et al., 2013). Lower frequencies were observed in both Norwegian Red and Ayrshire cows, ranging from 21% to 25% (Ødegård et al., 2013; Häggman et al., 2013). Coignard et al. (2013) demonstrated that the Montbeliarde breed has a higher overall health score compared to Holstein. However, no difference was observed in the frequency of lameness. The Montbeliarde breed is known for e.g. good health, fertility and longevity which is one of the reasons why this breed is used for cross-breeding with Holstein (Heins et al., 2012; Hazel et al., 2014). Frequencies and genetic parameters of claw disorders in Montbeliarde have not been reported to the best of our knowledge. Genetic parameters of claw disorders have been estimated in several dairy breeds such as Holstein (e.g. Van der Spek et al., 2013), Swedish Red (e.g. Buch et al., 2011), and Norwegian Red (Ødegård et al., 2013). Heritabilities for claw disorders estimated with a linear animal model in Holstein ranged from 0.01 to 0.14 (Van der Waaij et al., 2005; Van der Linde et al., 2010; Johansson et al., 2011; Van der Spek et al., 2013) and in Swedish Red from 0.01 to 0.07 (Buch et al., 2011; Johansson et al., 2011). Besides claw disorders also the need for trimming, the so-called “trimming status”, has been shown to be heritable (heritability of 0.09) in Holstein (Van der Spek et al., 2013). Less trimming is favorable and these results suggest that the need for trimming can be reduced by genetic selection. Knowledge on the genetic background of trimming status is limited and therefore confirmation in an independent population is needed. Aim of this research was to estimate frequencies and heritabilities for claw disorders and trimming status in Montbeliarde cows.

5.2 Materials and Methods

Data

The initial dataset contained 11,762 Montbeliarde cows. Cows with both parents unknown, with two different trimming records on the same date, or missing data on parity and lactation stage were removed. The final dataset contained 10,766 cows, of which 5,331 cows were present in the herd at time of trimming but were not offered for trimming and 5,435 cows were trimmed with a total of 10,233 trimming records. Repeated observations occurred within and across lactation; 52% of the trimmed cows were trimmed once, 25% was trimmed twice, and 23% was trimmed three times or more. Data concerning claw health were collected by professional claw trimmers during routine visits to 178 dairy farms in France between January 2007 and February 2013. The trimmed cows descended from 580 sires. Not all cows present in the herd during a routine visit of the claw trimmer were actually trimmed, as the farmer decided if and which cows were to be trimmed. Information on cows present in a herd at time of trimming on e.g. parity, stage of lactation and pedigree were available from milk recordings and national herd book registrations. Claw disorders analyzed in the current study were: double sole (DS), interdigital hyperplasia (IH), sole hemorrhage (SH), sole ulcer (SU), white line separation (WLS) and a combination of the infectious lesions digital dermatitis, heel horn erosion, and interdigital dermatitis (DER). The presence of claw disorders were scored as binary traits with score 1 (claw disorder present in at least one hind leg) or 0 (no claw disorder present). In addition, a binary trait (trimming status) was defined to indicate whether a cow was trimmed (score 1) or not (score 0). Claw disorders and trimming status were described in more detail in Van der Spek et al. (2013).

Statistical Analyses

The frequency of claw disorders was calculated as the number of trimmed cows with a specific claw disorder between 2007 and 2013 as a fraction of the total number of trimmed cows. Genetic parameters estimated for claw disorders were based on univariate analyses on trimmed cows only. Claw disorder records for untrimmed cows were set to missing. Genetic parameters estimated for trimming status were based on univariate analyses including trimmed and untrimmed cows. The following linear animal model was used:

$$Y_{ijklmn} = \mu + H_i + YS_j + P_k + L_l + Animal_m + PE_n + e_{ijklmn}$$

where Y_{ijklmn} is a claw disorder, μ is the overall mean, H_i is the fixed effect of herd i , YS_j is the fixed effect of year-season of trimming class j (each season consists of three consecutive months with spring = March – May), P_k is the fixed effect of parity class k ($k = 1, 2, 3$, and ≥ 4), L_l is the fixed effect of lactation stage at trimming class l ($l = 1$ to 10, group 1-9 are 50 days each, with the first group from 1-50 days, the second group from 50-100 days, etc. Cows with lactation stage ≥ 450 days were combined in group 10). $Animal_m$ is the random additive genetic effect of the m^{th} cow $\sim N(0, \mathbf{A}\sigma_a^2)$, where \mathbf{A} is the additive genetic relationships matrix among animals. The total pedigree comprised 23,278 animals. PE_n is the random permanent environment effect $\sim N(0, \mathbf{I}\sigma_{pe}^2)$ and $e_{ijklmno}$ is the random residual effect $\sim N(0, \mathbf{I}\sigma_e^2)$, where \mathbf{I} is the identity matrix. Heritability (h^2) was estimated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

and repeatability (r) was estimated as:

$$r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

where σ_a^2 is the additive genetic variance, σ_{pe}^2 is the permanent environmental variance and σ_e^2 is the residual variance. To quantify the importance of differences between herds on claw disorders and trimming status, additional analyses were performed using a model with herd included as a random effect. Analyses were performed using ASReml (Gilmour et al., 2009). Claw disorders were scored on a binary scale. Underlying the observed binary claw disorders there might be a continuous liability to develop claw disorders. By transforming observed heritabilities to the underlying scale, differences in claw disorder frequencies are taken into account (Gianola, 1982). Heritabilities were transformed to the underlying scale using the method as reported by Dempster and Lerner (1950).

5.3 Results

Frequency of claw disorders and trimming status

Frequencies of claw disorders in trimmed cows ranged from 9.4% for IH to 41.1% for SH (Table 5.1). From the trimmed cows, 72.9% had at least one of the claw

disorders between 2007 and 2013. SH, DER, and WLS were the most common claw disorders, whereas IH was the least common. Overall frequency of trimming status was 50%, indicating that half of the cows was trimmed at least once between 2007 and 2013. On average 46% of the cows was trimmed per herd per year.

Table 5.1. Claw disorder frequency in trimmed cows (N = 5,435) measured between 2007 and 2013.

Claw disorder	Abbreviation	Frequency (%)
Sole hemorrhage	SH	41.1
Infectious lesions	DER	33.3
White line separation	WLS	27.9
Double sole	DS	19.7
Sole ulcer	SU	13.6
Interdigital hyperplasia	IH	9.4
Any claw disorder		72.9

Systematic environmental effects

The effect of herd and year-season in the model was significant ($P < 0.01$) for all traits, parity was significant ($P < 0.05$) for all traits except DER and lactation stage was significant ($P < 0.05$) for all traits except IH and WLS. Herd explained 2.2% (IH) to 9.3% (DER) of the total variance for different claw disorders and 13.8% for trimming status. The frequency of SH and WLS was higher in autumn as compared to other seasons across years. For DER and DS, a clear trend was not found for season across years, the season with highest claw disorder frequency was different each year and often not significant. SU had highest frequency during summer (2007, 2008, and 2010) and spring (2011 and 2012). IH had highest frequency in summer (2007, 2008), autumn (2009, 2010), and winter (2011). Trimming occurred most in winter, followed by autumn and spring. The frequency of trimming in summer was significantly lower as compared to other seasons, indicating cows were least often trimmed during summer. Table 5.2 shows the least squares means for claw disorder frequencies in different parities. The frequency of WLS and trimming status increased with increasing parities. Frequency of WLS in parity 4 or higher was significantly higher than earlier parities and frequency in parity 1 was significantly lower as compared to later parities. The frequency of trimming status in each parity was significantly different from other parities, in other words the increase in frequency from one parity to the next is significant. The frequency of SH

was significantly higher in first parity as compared to later parities, whereas the frequency of IH was significantly lower in first parity as compared to later parities. The frequency of DS was significantly higher in parity 4 or higher. Figure 5.1 shows the least squares means for claw disorder frequencies in different lactation stages. SH had highest frequency from 50 to 150 days in lactation, SU from 50 to 200 days in lactation, DER from 50 to 150 and above 400 days in lactation, and trimming status from 50 to 100 days in lactation. DS, IH, and WLS occurred most at the end of lactation.

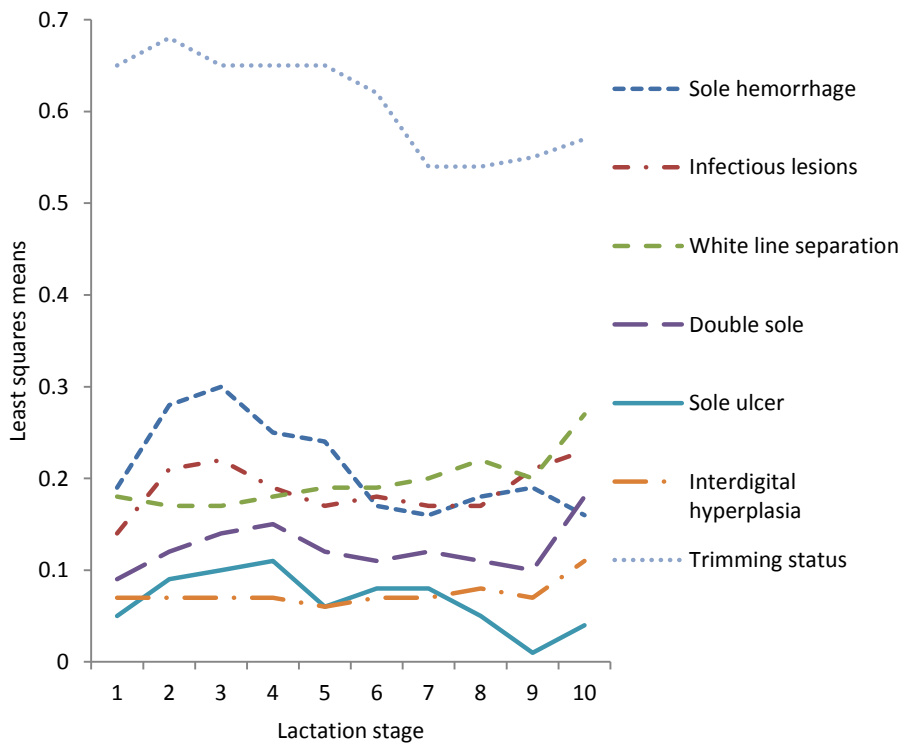


Figure 5.1. Least squares means for frequencies in different lactation stages (lactation stages are 50 days each; 1 = 0 to 49 days, 2 = 50 to 99 days, etc.) of claw disorders in trimmed cows and trimming status. Standard errors ranged between 0.01-0.04.

Table 5.2. Least squares means with standard errors between parentheses for frequencies in different parities of claw disorders in trimmed cows and trimming status.

Trait and abbreviation		Parity 1	Parity 2	Parity 3	Parity ≥ 4
Sole hemorrhage	SH	0.29 ^a (0.01)	0.23 ^b (0.01)	0.21 ^b (0.02)	0.22 ^b (0.01)
Infectious lesions	DER	0.18 (0.01)	0.19 (0.01)	0.19 (0.01)	0.20 (0.01)
White line separation	WLS	0.12 ^a (0.02)	0.17 ^b (0.02)	0.21 ^b (0.02)	0.26 ^c (0.02)
Double sole	DS	0.10 ^b (0.01)	0.11 ^b (0.01)	0.11 ^b (0.01)	0.16 ^a (0.01)
Sole ulcer	SU	0.09 ^a (0.01)	0.06 ^b (0.01)	0.07 (0.01)	0.08 (0.01)
Interdigital hyperplasia	IH	0.04 ^a (0.01)	0.08 ^b (0.01)	0.07 ^b (0.01)	0.09 ^b (0.01)
Trimming status		0.54 ^a (0.02)	0.58 ^b (0.02)	0.62 ^c (0.02)	0.66 ^d (0.02)

^{a,b,c,d} different superscripts within rows indicate a significant difference ($P < 0.05$)

Heritabilities and repeatabilities

Heritabilities and repeatabilities estimated for claw disorders were based on information from trimmed cows only. Table 5.3 shows heritabilities for claw disorders both on the observed and underlying scale. Estimated heritabilities on the observed scale ranged from 0.01 ± 0.01 to 0.09 ± 0.02 . Some claw disorders have very low heritabilities (e.g. DER), whereas other claw disorders have higher heritabilities (e.g. WLS). Heritabilities on the underlying scale were higher when compared to heritabilities on the observed scale and ranged from 0.02 ± 0.01 (DER) to 0.16 ± 0.04 (IH and WLS). Heritability for trimming status was higher than most claw disorders and estimated at 0.06 ± 0.02 on the observed scale and 0.11 ± 0.03 on the underlying scale. Repeatabilities differed between claw disorders and ranged from 0.02 ± 0.01 (SH) to 0.28 ± 0.01 (IH). The repeatability for trimming status was 0.27 ± 0.01 .

Table 5.3. The heritability on the observed (h_o^2) and underlying (h_u^2) scale and repeatability (r) with standard errors (SE) for claw disorders in trimmed cows and trimming status.

Trait and abbreviation		σ_p^2	r (SE)	h_o^2 (SE)	h_u^2 (SE)
Sole hemorrhage	SH	0.16	0.02 (0.01)	0.02 (0.01)	0.03 (0.01)
Infectious lesions	DER	0.13	0.03 (0.01)	0.01 (0.01)	0.02 (0.01)
White line separation	WLS	0.14	0.11 (0.01)	0.09 (0.02)	0.16 (0.04)
Double sole	DS	0.10	0.03 (0.01)	0.02 (0.01)	0.04 (0.02)
Sole ulcer	SU	0.08	0.13 (0.01)	0.03 (0.02)	0.09 (0.03)
Interdigital hyperplasia	IH	0.06	0.28 (0.01)	0.05 (0.02)	0.16 (0.04)
Trimming status		0.21	0.27 (0.01)	0.06 (0.02)	0.11 (0.03)

Genetic correlations of claw disorders and trimming status

Table 5.4 shows genetic correlations between claw disorders and trimming status. Genetic correlations were all positive, ranging from 0.10 (± 0.25) to 0.90 (± 0.31) but standard errors were rather high. A low genetic correlation was found for DER with trimming status (0.10), whereas high genetic correlations for SU and DS with trimming status were found (0.76 and 0.90 resp.).

Table 5.4. Genetic correlations (r_g) from bivariate analyses between trimming status and claw disorders in Montbeliarde cows.

Claw disorder		r_g (SE)
Interdigital hyperplasia	IH	0.65 (0.18)
Infectious lesions	DER	0.10 (0.25)
White line separation	WLS	0.50 (0.11)
Sole ulcer	SU	0.76 (0.18)
Double sole	DS	0.90 (0.31)
Sole hemorrhage	SH	0.24 (0.20)

5.4 Discussion

Frequencies of claw disorders and trimming status

Frequencies of claw disorders observed in Montbeliarde in the current study ranged from 9.4% (IH) to 41.1% (SH) in trimmed cows. In Holstein frequencies ranged from 0.1% (corkscrew claw, hardship groove) to 23.8% (DER) (Van der Spek et al., 2013), and from 0.6% (interdigital phlegmon) to 39.9% (SH) (Van der Waaij et al., 2005). A study in Swedish Red cows observed frequencies ranging from 4% (SU) to 25% (SH) (Buch et al., 2011). In Ayrshire cows frequencies ranged from 0.3% to 13.9% (Häggman et al., 2013) and in Norwegian red from 1.7% to 4.4% (Ødegård et al., 2013). SH is one of the most common claw disorders in the dairy breeds Holstein, Swedish Red and Ayrshire, equivalent to the Montbeliarde breed. The classification of infectious claw disorders digital dermatitis (DD), interdigital dermatitis (ID), and heel horn erosion (HE) differed between studies. In some studies they were recorded separately, in others as a combination of DD+ID, ID+HE, or DD+ID+HE. The different classifications complicate the comparison of results across studies. The frequency of trimmed cows having at least one of the observed claw disorders in at least one hind leg in our study was 72.9%. In Holstein, frequencies of 70% (Van der Waaij et al., 2005) and 54.8% (Van der Spek et al., 2013) have been found. In Norwegian Red the frequency of trimmed cows with at least one claw disorder was 21.3% in (Ødegård et al., 2013) and in Ayrshire cows

24.7% (Häggman et al., 2013). Norwegian Red cows seem to have less claw disorders as compared to Holstein and Montbeliarde. However, this difference could also result from different criteria for trimming cows or different scoring of claw disorders. In the study by Ødegård et al. (2013) most cows were trimmed by “other” claw trimmers, that lack certification. It might be that the quality of diagnosing claw disorders differs between these other claw trimmers and professional claw trimmers. As compared to Ødegård et al. (2013), the study by Johanssen et al. (2011) showed higher claw disorder frequencies in Red Dairy Cattle from Finland, Denmark and Sweden, these cows were trimmed by professional claw trimmers. Jersey cattle seemed to have less claw disorders as compared to Holstein and Red Dairy Cattle from the same study (Johanssen et al., 2011), but also as compared to most other studies. Montbeliarde seemed to have the highest frequency of claw disorders. However, it is important to keep in mind that not all cows were trimmed. On average 46% of the cows were trimmed per herd per year and most cows were kept in herds that trim less than 35% of the cows or 35-70% of the cows per year. In Holstein it was shown that most cows were kept in herds where 35-70% or more than 70% of the cows were trimmed per year (Van der Spek et al., 2013). The higher frequency in Montbeliarde may therefore be a consequence of the smaller proportion of trimmed cows. Montbeliarde has a higher health score compared to Holstein (Coignard et al., 2013). As a consequence, the farmer might be more strict in selecting cows for trimming. It may also be that claw disorders in Montbeliarde are generally less severe, such that claw trimmers score claw disorders even when they are hardly there.

Systematic environmental effects

Herd explained 2.2% to 9.3% of the total variance with regard to claw disorders. In a previous study (Van der Spek et al., 2013), similar herd effects were found in Holstein except for DER and WLS, where herd explained 18% and 25% respectively of the total variance. Highest claw disorder frequencies were found in autumn for SH, WLS, and later years for IH. Van der Spek et al. (2013) found similar results. Grazing has shown to improve hoof health (Somers 2005a,b) and might explain the seasonal differences in claw disorders. Bruijnis et al. (2010) observed in Holstein that claw disorders were more frequent at the end of the housing period (80%) as compared to the end of the grazing period (43%). Indicating a positive effect of pasturing on claw disorders during the summer months.

Least squares means for most claw disorders were higher for later parities. Increased claw disorder frequency for later parities was also found by Van der Waaij et al. (2005), Gernand et al. (2012), and Van der Spek et al. (2013). Van der

Spek et al. (2013) found a decrease in frequency of DER with increasing parities which was also found by Gernand et al. (2012). A study by Chapinal et al. (2013) found an increase of DER for increasing parities and in the current study a small, non-significant increase of DER was observed for increasing parities. Frequency of SH decreased with parity as was also found by Van der Spek et al. (2015). The current study showed a higher frequency of SH and DER in the period of 50 to 150 days in lactation and for SU from 50 to 200 days in lactation. In Norwegian Red cows, highest frequencies of SU and SH were observed during 90 to 150 days of lactation (Ødegård et al., 2013). The frequencies of other claw disorders (e.g. HE, ID, DD, WLS) increased slightly during lactation (Ødegård et al., 2013). Van der Spek et al. (2013) observed similar results in Holstein.

Genetic parameters for claw disorders

Estimated heritabilities on the observed scale ranged from 0.01 (DER) to 0.09 (WLS) which is in line with previous studies (e.g. Van der Waaij et al., 2005; Buch et al., 2011; Johanssen et al., 2011; Van der Spek et al., 2013). Differences in frequency of binary traits is known to influence the level of heritability on observed scale. This effect can be taken into account by transforming observed heritabilities to heritabilities on the underlying scale (Gianola, 1982). An alternative for transformation is estimating heritabilities on the underlying scale directly by using a threshold model. We observed that using a threshold model or transformation from observed to underlying scale using the method as proposed by Dempster and Lerner (1950) resulted in similar heritabilities (unpublished results). In the current study, heritabilities on the underlying scale after transformation ranged from 0.02 (DER) to 0.16 (IH, WLS). Other studies in different dairy breeds, Holstein, Ayrshire and Norwegian Red cows, observed comparable ranges of heritabilities on the underlying scale for claw disorders as observed in the current study. On the underlying scale, heritability for WLS appeared to be slightly higher than heritabilities measured in Holstein (Gernand et al., 2013; Häggman and Juga, 2013; Van der Spek et al., 2013), Ayrshire cows (Häggman et al., 2013) and Norwegian Red cows (Ødegård et al., 2013). Heritability for IH seems to be lower as compared to Gernand et al. (2013) and Van der Spek et al. (2013). Heritabilities for DER seemed to be lower in Montbeliarde when compared to Holstein (Van der Spek et al., 2013). Heritability of SH and SU seemed to be lower than Norwegian Red (Ødegård et al., 2013) but comparable to heritabilities measured in Holstein (e.g. Häggman and Juga, 2013). Heritability for SU seemed to be lower than in Ayrshire cows (Häggman et al., 2013).

Repeatabilities observed in the current study are comparable with results in Holstein cows (Van der Linde et al., 2010; Van der Spek et al., 2013). Studies in Holstein and in Montbeliarde both indicate high repeatabilities for IH. IH is excessive growth of skin and tissue between the claws and needs to be removed surgically. The chance that this claw disorder will return is high (Enevoldsen and Gröhn, 1991). Also, IH is mostly not treated which explains the high repeatability. The repeatabilities measured for the other claw disorders in this study are generally low, indicating that having repeated observations increases accuracy of estimated breeding values.

Genetic parameters for trimming status

Trimming status was found to be heritable in Montbeliarde, which confirms the result found in a previous study in Holstein (Van der Spek et al., 2013). The cows in both studies were kept under similar conditions and were trimmed by the same claw trimmers. The heritability measured for trimming status on the observed scale (0.06) and the heritability on the underlying scale (0.11) were slightly lower than heritabilities in Holstein (0.09 on the observed and 0.14 on the underlying scale). Genetic correlations between claw disorders and trimming status were all positive and generally higher, but based on standard errors not significantly, as compared to the estimates in Holstein (Van der Spek et al., 2013). This study confirms that trimming status is an interesting trait for improving claw health.

5.5 Conclusion

Aim of this study was to estimate heritabilities for claw disorders and trimming status in Montbeliarde cows. In addition, genetic correlations between the different claw disorders and trimming status were estimated. The frequency of claw disorders in trimmed Montbeliarde cows was high, especially for SH, DER, and WLS. Results confirm earlier findings that claw disorders are heritable. Most heritabilities estimated for claw disorders on the observed and underlying scale were comparable to heritabilities estimated in studies using other dairy breeds (Holstein, Ayrshire, Norwegian Red and Swedish Red). Also, trimming status was found to be heritable, confirming the result found in Holstein. This study shows that trimming status is an interesting trait to be included in genetic selection programs to improve claw health.

5.6 Acknowledgements

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6

General discussion

6.1 Introduction

Consumers are becoming more concerned about animal health and welfare, especially in developed countries. This results in an increasing demand for animal products produced in a more animal friendly way. Reduced animal health also influences farm profitability. Therefore, improving health of livestock is important from a welfare but also from an economical point of view. A trait in dairy cattle affecting welfare and farm profitability is claw health. Several studies have indicated a high frequency of claw disorders with around 70% of trimmed cows having at least one claw disorder in the time period measured (Van der Waaij et al., 2004; Van der Linde et al., 2010). However, claw disorders consist of a broad range of different disorders where some cause hardly any visible health problems for the cow whereas others cause severe health problems. Manske et al. (2002) showed that 5% of the cows with claw disorders (72%) were clinically lame. Bruijnis et al. (2012) showed that clinical interdigital phlegmon and sole ulcer are most painful, whereas subclinical white line disease and sole hemorrhage are least painful. Taking the duration of claw disorders next to pain into account, Bruijnis et al. (2012) found the highest impact on welfare for clinical hygiene related claw disorders (interdigital hyperplasia, digital dermatitis, and interdigital dermatitis combined with heel horn erosion). When also incidence of claw disorders is taken into account, subclinical claw disorders have a higher impact on welfare than clinical claw disorders. Subclinical sole hemorrhage having the highest welfare impact on herd level and clinical interdigital phlegmon the lowest impact (Bruijnis et al. 2012). As claw disorders occur frequently in dairy cows and affect cow welfare and farm profitability, breeding organizations are interested in improving claw health. Aim of this thesis was to gain insight in the genetic background of claw disorders in dairy cattle. In order to quantify opportunities for selection, genetic parameters were estimated for claw disorders (Chapter 2). Although heritabilities were low, genetic selection to reduce frequency for claw disorders is feasible. I also defined a new trait, trimming status, which reflects the need for trimming. Trimming status is heritable and therefore genetic selection for cows that need less trimming is feasible. Major genes influencing susceptibility to claw disorders or trimming status were not identified (Chapter 3). However, genomic selection may still be a good opportunity to reduce frequency of claw disorders. In this general discussion I will focus on alternative claw disorder phenotypes which might result in more accurate recording of claw disorders and higher heritabilities. I will also compare theoretical accuracies for traditional selection and theoretical and realized accuracies for genomic selection to reduce frequency of claw disorders,

and estimate expected selection response for claw disorders of alternative breeding schemes.

6.2 Phenotyping claw disorders

6.2.1 Current situation

Trimming records in our study were collected by professional claw trimmers on regular visits to farms. Farmers contact the claw trimmer whenever they find it necessary and the farmer decided which cows were to be trimmed. The accuracy of diagnosing claw disorders depends on the farmer and the claw trimmer. The farmer decides which cows will be trimmed and most probably selects cows with excessive horn growth or claw disorders. Likely, some cows in need of trimming due to claw disorders will not be identified as only 5% of the cows with claw disorders is clinically lame (Manske et al., 2002). Claw trimmers on the other hand, may misdiagnose an animal to be healthy or affected. Scoring claw disorders is subjective (Greenough, 1987) and quality depends on how skilled the claw trimmer is (Whay, 2002). Bishop and Woolliams (2010) showed that incorrectly diagnosing an animal with a disease or incorrectly diagnosing an animal to be healthy leads to an underestimation of the heritability. Incomplete data recording (i.e. not all affected animals are recorded) reduces heritability as well and the underestimation of heritability is greater when disease frequency is lower (Bishop and Woolliams, 2010). Besides incorrect diagnoses, scoring claw disorders is labor intensive and expensive. When accurate phenotypes for a specific trait are difficult or expensive to obtain, sometimes indicator traits might provide a good alternative. These indicator traits must be heritable, have a moderate to high genetic correlation with the trait of interest, and should be easier and less expensive to obtain. For claw disorders, claw and leg conformations scores have been proposed as possible indicator traits. Claw and leg conformation scores are collected on a routinely basis for genetic improvement programs. Genetic correlations between claw disorders and feet and leg conformation traits are low to moderate ranging from -0.58 to 0.41 (Van der Linde et al., 2010). Claw and leg conformation scores are informative but cannot replace direct scores of claw disorders (Swalve et al., 2004; Uggla et al., 2008). Swalve et al. (2004) showed that the accuracy of selection decreased when claw and leg conformation scores were used instead of direct scores on claw disorders.

The focus of breeding programs is on measuring and reducing claw problems. In Chapter 2 and 5 it was shown that the need for trimming, the so-called “trimming status”, is heritable as well. Trimming status may provide an interesting way to

select for a type of claw that requires less trimming and has less claw disorders. Trimming status is a bulk trait containing information on multiple claw disorders, but also new information such as excessive horn growth which is currently not considered.

In short, claw disorder heritabilities may be underestimated due to selective and subjective scoring which is prone to mistakes and reasons to trim the claws of certain cows, other than claw disorders, are not considered. Therefore, aim of this section is to investigate possibilities for more precise phenotyping of claw health traits and investigate possible indicator traits.

6.2.2 Definition of phenotypes

Claw disorders

Scoring performed by claw trimmers is a subjective method to identify claw disorders. Variability between claw trimmers exists, although it can be reduced with training (Manske et al., 2002; Thomsen and Baadsgaard, 2006; Capion et al., 2008). More precise scoring of claw disorders is expected to reduce noise and increase heritability. An interesting result was found by Schöpke et al. (2015) using multiple classes to score digital dermatitis instead of a binary scale. Schöpke et al. (2015) reported a substantial increase in heritability of digital dermatitis by subdividing digital dermatitis in 6 development stages according to a previous proposed M-scale (Döpfer et al., 2012). In the M-scale M0 indicates no lesion, M1 a small (<2cm) early lesion, M2 an active lesion (>2cm), M3 a healing lesion with scald, M4 a chronic lesion, and M4.1 a chronic active lesion. Schöpke et al. (2015) found heritabilities varying from 0.19 (± 0.11) to 0.52 (± 0.17) estimated with a threshold model when different classes of the M-scale were used or combined. These estimates are substantially higher as compared to heritabilities for digital dermatitis estimated in previous studies. For instance, König et al. (2005) found a heritability of 0.07 and Häggman and Juga (2013) found a heritability of 0.13. Schöpke et al. (2015) found a heritability of 0.19 when classes of the M-scale were combined to obtain a binary scale, which is already higher as compared to previous studies suggesting that the increased heritability is not only caused by using more classes. Possible reasons, besides multiple classes, for the high heritabilities could be the single, highly experienced observer scoring all cows, the use of a single herd, and scoring cows at least three times in a 15 month period. On the other hand, studies using multiple classes to score claw disorders, e.g. Van der Linde et al. (2010) scored several claw disorders on a severity scale from 0 to 3, did not find higher heritability estimates for claw disorders as found in studies using a binary

scale. Scoring claw disorders using multiple classes as opposed to present or absent, can lead to certain classes containing only a few number of observations. For example, Buch et al. (2011) scored claw disorders on a scale of 0 to 2. Due to the few number of observations in class 2, they decided to analyze claw disorders as binary traits combining class 1 and 2. A study performed on a disease trait in horses showed that heritabilities estimated on the underlying scale using either a binary scale or a scale with multiple classes from 0 to 2 resulted in equal heritability estimates (Schurink et al., 2009). More research is needed to confirm the interesting heritability estimates found by Schöpke et al. (2015).

Trimming status

The farmer decides when and which cows will be trimmed. This selective data recording is considered to be a problem and therefore several studies only used data where the majority of the cows, and ideally all cows, are being trimmed. In Chapter 2 and 5 we showed that there is value in using the information about which cows are selected by the farmer for trimming. In Chapter 2 it was shown that 45% of the trimmed cows do not have any claw disorders, but still the farmer decided to trim these cows. A reason for the farmer to decide on trimming a cow could be excessive horn growth. Therefore, trimming status is a valuable trait providing additional information on the need for trimming besides claw disorders. This information will be lost when standard practice is to trim all cows (unless excessive horn growth is scored). Currently trimming status is not considered in breeding programs. A higher need for trimming is unfavorable as it is expensive and besides excessive horn growth often an indication of claw health problems. Laminitis has shown to be associated with increased horn growth (Greenough et al., 1990; Vermunt, 1990). In Chapter 2 of this thesis it was shown that trimming status is genetically correlated to claw disorders. Genetic correlations vary from approximately 0.20 to 0.65 (± 0.10). Selection for a reduced need for trimming, results in a maintenance and problem free claw. The ultimate goal would be to breed for “trim-free” cows. Trimming status, indicating for each cow in the herd whether it needs trimming (score 1) or not (score 0), should be included as a novel index trait.

6.2.3 Indicator traits for claw health

Scoring claw disorders is expensive and prone to mistakes due to the subjective way of scoring, even more so if there are more severity classes. Besides scoring claw disorders, also the decision by the farmer of which cows should be trimmed is

subjective. Indicator traits that are easier to measure may be a way to obtain objective scores for claw health traits with higher heritability as compared to subjective scores of direct claw disorder measurements. In this section, possible indicator traits are proposed and discussed for claw health traits.

Pressure plates

Lame animals redistribute their weight among sound legs in order to bear less weight on the affected leg (Pastell and Kujala, 2007), often resulting in visible difficulties while walking. Pressure plates measure ground reaction force and the maximum load applied during stance time is the peak vertical force (N/kg) (Lascelles et al., 2006). The weight distribution on a specific leg is the proportion of the total peak vertical force attributable to this leg (Kim et al., 2011). The weight distribution can be recorded as a quantitative trait and it can be measured over a long time period (Chapinal et al., 2009). A system for automatic lameness detection using ground reaction force measurements is already commercially available (StepMetrix™, BouMatic, Madison, WI, US). Bicalho et al. (2007) showed in a field trial that the model used by the system correctly identified non-lame animals (i.e. high specificity) but misclassified lame animals (i.e. low sensitivity). Liu et al. (2009) showed an improved algorithm for the system, thereby substantially increasing the sensitivity to detect lame animals. Almeida et al. (2007) showed a lower peak vertical force for animals with digital dermatitis. They also showed that animals undiagnosed using a subjective lameness scoring system, were detected with the pressure plate. However, Bicalho et al. (2007) showed that a higher specificity for a higher locomotion score was obtained, suggesting that mostly severe cases of lameness can be detected. Severe lame cows can also be easily detected by the farmer due to the clear impaired gait. Bruijnjs et al. (2010) showed that besides clinical claw problems, also subclinical claw problems cause economic losses. It is not clear whether these subclinical cases can be detected with a pressure plate. More research is needed to investigate whether pressure plates can identify subclinical lame cows. Besides, heritability for the weight distribution measured by pressure plates and genetic correlations with claw health traits have not been estimated. Therefore, it is too early to conclude if this method is suitable for genetic evaluation of claw health. Next to that, it is expensive to equip all herds with a StepMetrix system. The cost for a complete system including electronics and software is €30,000 (Beunk, 2010). Specific test herds could be selected to install the equipment and herds that have already such a system installed could also be used as test herds in order to estimate genetic parameters and investigate if subclinical claw disorders can be detected.

Infrared thermography

Infrared thermography (IRT) is an easy method to evaluate surface temperature of the claw and is non-invasive. Increased blood flow as a response to injury and inflammation in extremities causes the temperature to rise. For mastitis, IRT has been used to investigate inflammation (Berry et al., 2003). Several studies have found a significant increase in claw temperature in cows with a claw disorder (Nikkhah et al., 2005; Alsood and Büscher, 2012; Main et al., 2012; Stokes et al., 2012). A rise in temperature was identifiable weeks before a cow shows visible symptoms of lameness (Wood et al., 2014). Stokes et al. (2012) showed that temperature measurements were best performed without washing the claws as washing the claws affects the temperature. Wood et al. (2014) found an increased temperature for claws with claw horn lesions (bruising, sole ulcer, sole separation, white line disease and injuries to the sole). They could not distinguish based on temperature between specific claw lesions. For soft tissue lesions (heel horn erosion, interdigital growth, and interdigital dermatitis), an increase of temperature was not found and it was argued that this was due to the low number of cows with a soft tissue lesion (Wood et al., 2014). On the other hand, Main et al. (2012) were able to find a significant increase in temperature for soft tissue lesions. However, heritabilities of claw health traits diagnosed with IRT and genetic correlations with claw disorders scored by claw trimmers have not been estimated so far. Application of infrared thermography on farm is limited to prioritizing animals to be trimmed. Diagnosing specific claw disorders still have to be done by picking each feet up.

Reliable identification of cows with claw disorders is possible when automated systems are used, monitoring each cow frequently to identify normal claw temperature. An automated thermal imaging device to detect mastitis, the CaDDi mastitis, has been developed by Agricam and FLIR Commercial Systems (Linköping, Sweden). A similar system could be developed for detection of claw disorders, but is rather expensive. A single camera costs more than €10,000,- and two cameras are needed plus the software. Main et al. (2012) found a sensitivity and specificity of 78% using a low-cost handheld thermometer. Identification with a hand-held infrared thermometer can be done easily (feet do not need to be picked up), at an early stage, and at relatively low cost of around €50 in an electronic store. However, the use of a hand-held infrared thermometer is very labor intensive as cows should be measured several times a week and all measures have to be written down and digitalized for analyses. Therefore, it is unlikely that farmers will adapt this technique to collect claw health data.

Pedometer / activity monitor

Cows with claw disorders tend to walk less and spend more time lying down (Juarez et al., 2003; Walker et al., 2008). Other behavioral changes seen in lame cows are for example shorter grazing time (Hassall et al., 1993) and consuming feed faster (Proudfoot et al., 2010). Proudfoot et al. (2010) showed that behavioral changes are associated with claw horn disorders not visible yet. A pedometer attached to the leg of the cow can provide valuable information on reduced activity to use as an indication of claw health problems. Pedometers are used as an aid in fertility to detect estrus (increased levels of activity) as well. With a pedometer, a large amount of data can be collected. A deviation of the normal value, which can differ for each cow, can be assessed for each cow for activity traits (e.g. number of steps). O'Callaghan et al. (2003) found a significant association between claw disorders and activity measured with a pedometer. Miekley et al. (2012) showed a sensitivity of 50% and specificity of 80% using pedometers. However, genetic relationships between reduced activity and claw health traits still need to be investigated. Also, more research is needed to investigate whether subclinical claw disorders are detected as well. Investment costs range between approximately €11,000,- and €28,000,- (Lang, 2013) but farmers may already have a system with activity measurements in place for fertility, which could be used for detection of decreased activity as well.

Conformation scoring

Conformation scores are routinely collected and have shown to be an indicator trait for claw disorders. However, conformation scores cannot replace direct scores for claw disorders as explained in 6.3.1. Genetic correlations of claw and leg conformation scores with trimming status are unknown. However, a negative correlation between hoof length and hoof angle was found by e.g. Hahn et al. (1984), Politiek et al. (1986), and Distl et al. (1990). Hoof length may be related to excessive horn growth and thereby trimming status. Therefore, it is likely that trimming status is genetically correlated to at least some of the conformation scores but needs to be investigated. However, as conformation scores cannot replace direct scores for claw disorders it is unlikely they can replace trimming status.

Measuring the amount of horn

Trimming status is a measure for the need for trimming. A more precise measure would be the amount of horn that is trimmed from the claws. Horn amount can be

weighted at time of trimming and is an indicator trait for excessive horn growth. Horn that is trimmed from the claw can be weighted objectively with a scale, but horn needs to be washed first to remove dirt. Often claw trimmers use a grinder to trim the cows in the stable. It might not be easy to collect and clean the horn that is trimmed from the claw and it is labor intensive. Horn growth is also influenced by many other factors (Vermunt and Greenough, 1995), besides a genetic background. For example, young cows have a faster horn growth rate than older cows (Glicker and Kendrick, 1977). Tranter and Morris (1992) showed a seasonal influence on horn growth, with faster growth during summer. Manson and Leaver (1988) showed an influence of nutrition on horn growth and Hahn et al. (1986) showed an influence of floor surface. Claw trimming has shown to increase horn growth and decrease horn wear (Manson and Lever; 1989).

6.2.5 Conclusion

Currently there is no convincing evidence that alternative methods which objectively score specific claw disorders will improve heritability estimates and breeding value estimation. Therefore, the best option is to continue scoring claw disorders in the same way as currently being done.

6.3 Accuracy of genomic selection

Genetic improvement of claw health based on own performance is not easy as claw disorder heritabilities are low. Large progeny groups are needed for accurate selection which takes time, thereby increasing the generation interval. Claw disorder phenotypes become available after the cow has started to produce. In order to select animals earlier in life, genomic selection is a promising approach. Especially for low heritable traits genomic selection has shown to be of interest (Hayes et al., 2009; Calus, 2010). Aim of this section is to look at the accuracy of estimated breeding values for claw disorders based on conventional progeny testing and genomic selection. First, the theoretically expected accuracy for progeny testing and genomic selection will be compared. Next, the accuracy for genomic selection will be estimated based on real data. In this way it is possible to investigate how well theory predicts the realized accuracy of genomic selection.

6.3.1 Theoretical expectations

Conventional selection

In conventional breeding programs, phenotypic information is combined with pedigree information to estimate breeding values (EBV). In dairy cattle selection of bulls is based on progeny testing. Accuracy (r_{ih}) of selection in bulls based on progeny information can be calculated as follows:

$$r_{ih} = \sqrt{\frac{\frac{1}{4}nh^2}{1 + \frac{1}{4}(n-1)h^2}}$$

Where n is the number of daughters and h^2 is the heritability of the trait. Assuming each bull has 25 daughters with claw disorder records ($n = 25$) and a claw disorder heritability (h^2) of 0.05, the accuracy is 0.49. When a bull has 100 daughters, accuracy increases to 0.75. A progeny group of 25 may not seem very large as in general many more female offspring are produced per young bull, but for claw disorders 25 records is substantial. Figure 6.1 shows expected accuracies of estimated breeding values for different numbers of progeny and different heritabilities.

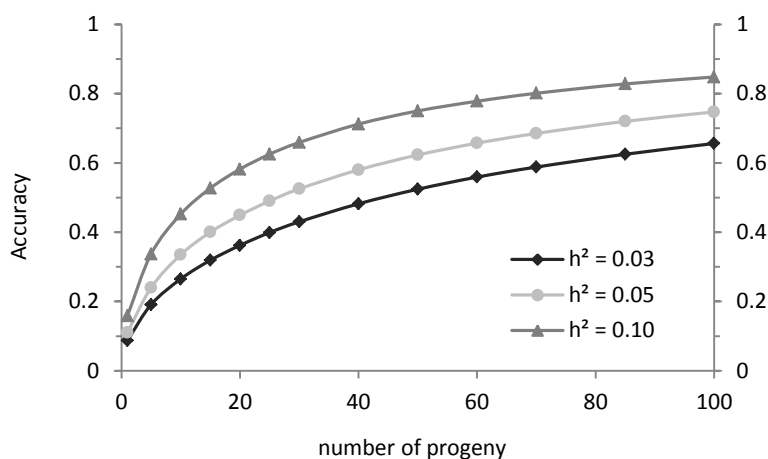


Figure 6.1. Accuracies for estimated breeding values based on progeny testing with different number of progeny and different heritabilities.

Genomic selection

Cows and bulls can be genotyped for thousands of single nucleotide polymorphisms (SNP) at relatively low cost. This has enabled the use of genomic selection (Meuwissen et al., 2001; Goddard and Hayes, 2009). In order to

implement genomic selection, SNP effects have to be estimated in a reference population. Animals in the reference population are genotyped and phenotyped. Estimated marker effects from the reference population are used to predict genomic estimated breeding values (GEBVs) for selection candidates. The selection candidates are animals that usually only have a genotype and no phenotype available. Using the formula in Daetwyler et al. (2013), we can calculate the theoretically expected accuracy (r_{ih}) for GEBVs for a reference population consisting of progeny tested bulls:

$$r_{ih} = \sqrt{\frac{N_p r^2}{N_p r^2 + M_e}}$$

and

$$M_e = \frac{2N_e L}{\ln(4N_e L)}$$

Where N_p is the size of the reference population, N_e is effective population size, L is the length of the genome, r^2 is the reliability of progeny testing, and M_e is the effective number of chromosome segments.

Assuming a reference population of 1,000 bulls with a genotype and EBV for claw disorders, effective population size of 100, length of the genome is 30 Morgan, reliability for progeny testing of 0.49^2 (which is based on 25 daughters and claw disorder heritability of 0.05), then accuracy for GEBVs is 0.52. The importance of the size of the reference population, especially with low heritable traits, is illustrated in Figure 6.2. With 5,000 progeny tested bulls in the reference population a high accuracy (0.81) for GEBVs can be obtained, but each bull must have 25 daughters and therefore this requires 125,000 cows with phenotypes on claw disorders. An increase from 25 to 100 daughters per progeny tested bull in the reference population increases accuracy for GEBVs from 0.81 to 0.90. The break-even point at which the accuracy of progeny testing with 25 progeny per bull ($r_{ih} = 0.49$) is equal to the accuracy of GEBVs, is at 840 bulls in the reference population (for a claw disorder heritability of 0.05).

In comparison, a reference population of cows can also be considered when the number of bulls is limited. Replacing r^2 with h^2 in the formula from Daetwyler (2013), the accuracy of GEBVs with a cow reference population can be calculated. With 4,038 cows in the reference population, an accuracy of 0.49 for GEBVs is estimated for a h^2 equal to 0.05.

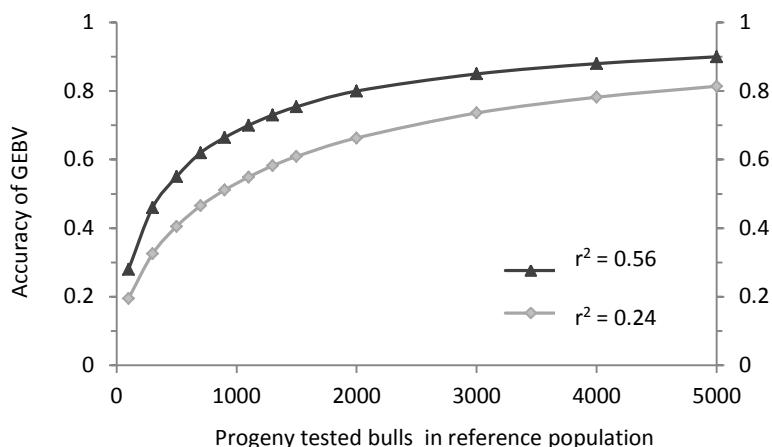


Figure 6.2. Accuracies for genomic estimated breeding values (based on Daetwyler et al., 2013) with different number of bulls in the reference population and a reliability (r^2) for progeny testing of 0.56 (corresponding to a heritability of 0.05 and a progeny group of 100) and of 0.24 (corresponding to a heritability of 0.05 and a progeny group of 25).

6.3.2 Real data

In the previous section, accuracies were calculated based on theory. In this section, accuracies will be estimated based on real data in order to investigate how well we theoretically can predict the accuracy for genomic breeding values. It is important to compare theoretical and realized accuracy as it is difficult to predict the exact values for N_e and thereby M_e used in theoretical predictions (van der Werf et al., 2014). Also, in theoretical predictions assumptions are made that GEBV accuracy results from SNP capturing the QTL effect. However, SNP may also capture family relationships resulting in a higher accuracy (Habier et al., 2007).

Leave-one-out cross-validation was performed to predict GEBVs for bulls and to calculate the accuracy of the predicted GEBVs. Data consisted of 71 genotyped bulls with at least 25 daughters with claw disorder phenotypes. Quality control of the genotypes was explained in Chapter 3. Only 25 daughters were used for each bull, if a bull had more daughters these were randomly removed to be able to calculate accuracies. Phenotypes of the 25 daughters were used to calculate Daughter-Yield-Deviations (DYD) for the bulls. DYD were calculated as the average phenotype (P^*) of the 25 daughters where P^* were cow phenotypes adjusted for systematic environmental effects. Variance components for the bulls with 25

daughters were estimated in ASReml (Gilmore et al., 2009) using a linear animal model with a random animal and residual effect.

Leave-one-out cross-validation was used with 70 bulls with genotype and phenotype in the reference population. The DYD of a single bull (71st) was removed from the data in order to mimic a situation in which a bull does not have any phenotype (or daughter information) available and GEBV of this bull was predicted. This was repeated for each bull; 71 times. BLUPf90 packages (Misztal et al., 2013) were used to estimate genomic breeding values (GEBV) using the G-matrix. The model was:

$$Y_i = \mu + Animal_i + e_i$$

Where Y is the DYD for a claw disorder, μ is the overall mean and $Animal_i$ is the random additive genetic effect of the i^{th} bull $\sim N(0, \mathbf{G}\sigma_a^2)$, where \mathbf{G} is the genomic relationship matrix among bulls and σ_a^2 is the genetic variance. The random residual effect is denoted by $e_i \sim N(0, \mathbf{I}\sigma_e^2)$, where \mathbf{I} is an identity matrix and σ_e^2 is the residual variance.

The correlations between DYD and GEBV were calculated and ranged from 0.16 to 0.36 depending upon claw disorder (Table 6.1). Dividing by the square root of the heritability for DYD gives the realized accuracy of GEBV. Accuracies for GEBV for the different claw disorders are shown in Table 6.1. Accuracies for GEBVs ranged from 0.24 to 0.45. Ødegård et al. (2015) reported a correlation of GEBV with deregressed proofs of 0.32 for a combination of hygiene-related claw disorders and 0.33 for a combination of laminitis-related claw disorders. These results seem to be in line with results found in the current section, however, it is difficult to compare both studies as the accuracy was not given and bulls had a different number of progeny with a minimum of 30 daughters.

Table 6.1. Correlation between daughter-yield-deviations (DYD) and genomic breeding values (GEBV) estimated using leave-one-out cross-validation and realized accuracy calculated as the correlation divided by the square root of the heritability for DYD.

Claw disorder	Correlation GEBV - DYD	Realized accuracy
Double sole (DS)	0.22	0.31
Infectious lesions (DER)	0.31	0.35
White line separation (WLS)	0.36	0.41
Sole hemorrhage (SH)	0.16	0.29
Sole ulcer (SU)	0.19	0.24
Interdigital hyperplasia (IH)	0.32	0.45

Theoretical expected accuracy for genomic selection, based on Daetwyler et al. (2013), range from 0.11 to 0.22 for claw disorder heritabilities ranging between 0.02 and 0.14. Realized accuracies are higher than theoretically expected. From Figure 6.3 it can be seen that an increase in expected accuracy corresponds generally to an increase in realized accuracy. Figure 6.3 also shows that realized accuracies are higher as compared to expected accuracies based on theoretical calculations.

Besides the accuracy, it is also important to investigate a possible bias of the breeding values. The regression coefficient of DYD on GEBV ranged from 0.5 to 1.2. A regression coefficient of 1 indicates no bias and based on the standard errors of the regression coefficients, none of the estimates differed significantly from 1. Christensen et al. (2011) reported similar bias estimated for GEBV for production traits in pigs and Luan et al. (2009) reported similar bias estimates for GEBV for various traits in Norwegian Red cattle. This shows that the difference in theoretically expected and realized accuracy cannot be explained by bias in GEBV.

Van der Werf et al. (2014) compared expected and realized accuracy for genomic selection in sheep. They also found for some traits a higher realized accuracy than theoretically expected. In dairy cattle, Hayes et al. (2009) showed that realized accuracies in Holstein and for some traits in Jersey were slightly lower than theoretically expected. For some other traits in Jersey the realized accuracy was higher than theoretically expected (Hayes et al., 2009). These reported accuracies were not adjusted for reliability of the phenotype. After adjustment, accuracies were found to be in better agreement for Holstein. For some traits realized accuracies were higher than theoretically expected after the correction (Hayes et al., 2009). Theoretical prediction of accuracy for genomic selection assumes unrelated individuals in the reference population (Daetwyler et al., 2013). In our case, there were family relations between the reference population and validation animals. The accuracy of the GEBV can result in a large part from genetic relationships between the reference population and validation population picked up by the SNP (Habier et al., 2007; Wientjes et al., 2013).

6.3.3 Conclusions

Accuracy of traditional selection for claw disorders based on progeny testing is low when bulls have a limited number of phenotyped daughters. For a heritability of 0.05 and 25 progeny per bull, the accuracy of traditional selection is 0.49. With genomic selection, 840 progeny tested and genotyped bulls are needed in the

reference population to obtain the same accuracy. The bulls in the reference population should have 25 daughters with phenotypes each, implying a total of 21,000 phenotyped cows. Accuracy for progeny testing increases to 0.75 when bulls have 100 daughters each and heritability is 0.05. For genomic selection, 1,500 bulls are needed (with 100 daughters each) in the reference population to obtain the same accuracy. A high accuracy (0.81) for genomic selection can be obtained with a reference population of 5,000 bulls genotyped, each with 25 daughters phenotyped for claw disorders and for a claw disorder heritability of 0.05. Obtaining such a large number of progeny tested bulls may not be feasible, instead, genotyped cows might be added to the reference population, as there are more cows available. Realized accuracies for genomic selection were calculated using a reference population of 70 bulls. The 70 bulls had a genotype and a daughter-yield-deviation based on 25 daughters. The realized accuracy of genomic selection varied from 0.24 to 0.45 for different claw disorders. Theoretical expected accuracy was lower and ranged from 0.11 to 0.22 for genomic selection. The difference between expected and realized accuracy was due to family relationships between reference population and validation animals.

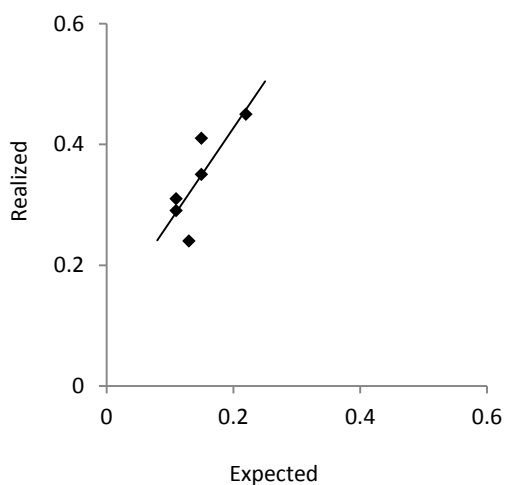


Figure 6.3. Accuracy of genomic estimated breeding values (GEBV) from cross-validation for claw disorders.

6.4 Breeding program to reduce claw disorders

In Chapter 2 genetic variation in susceptibility for claw disorders was found. This indicates there are possibilities to reduce the incidence of claw disorders by genetic selection. It also indicates that ignoring claw disorders as a trait in the breeding goal, likely results in increased incidence of claw disorders which I will explore further in this section. As heritabilities are low and phenotypes are only collected on females, genomic selection is a promising way to reduce the incidence of claw disorders. In section 6.3 I showed that the accuracy of genomic selection based on a reference population of 70 sires (with a DYD based on 25 daughters) is lower as compared to traditional EBV based on progeny testing sires (with 25 daughters). However, the generation interval for progeny information to become available is longer as compared to genomic information. Selection response increases considerably when based on genomic information instead of progeny information due to the shorter generation interval (Meuwissen et al., 2001). Selection response for claw disorders based on either progeny information or genomic information should be estimated to identify which information source results in a higher selection response.

In this section the selection response for claw disorders will be compared, using either progeny information or genomic information. A breeding program is assumed aimed at improving claw health as the only breeding goal. A single claw disorder was used as an index trait for the breeding goal. Selection was performed in bulls only. Response to selection is calculated using SelAction (Rutten et al., 2002). In SelAction a population with discrete generations and consisting of 20 sires and 200 dams was simulated. One-stage selection was performed with a selected proportion of 10% in bulls and 99.9% in dams (mimicking no selection in dams). Heritabilities and phenotypic variances for claw disorder estimated in Chapter 2 were used as input parameters. In text box 6.1, a summary of input parameters for the simulation are given.

Three scenarios are investigated. In the first scenario, bulls have progeny information on 25 daughters available and traditional selection based on pedigree BLUP and progeny information is performed. In the second scenario, bulls are selected based on genomic information as the only information source. In SelAction, genomic information was modelled as a separate trait (Schrooten et al., 2005) with a heritability of 0.99 and a genetic correlation with the claw disorder equal to the accuracy of genomic selection (estimated in 6.3.2). The phenotypic correlation was equal to the accuracy multiplied by the square root of the claw disorder heritability. The phenotypic variance was equal to the accuracy squared

multiplied by the additive genetic variance of the claw disorder. See text box 6.1 for further details. Finally, in the third scenario, a breeding goal aiming to improve milk production and claw health was assumed. The index trait for claw health was sole ulcer and for milk production was protein yield (kg). Progeny information of 25 daughters was available for sole ulcer and of 100 daughters for protein yield. Selection response was calculated for including only sole ulcer as an index trait, only protein yield, or sole ulcer and protein yield combined.

6.4.1 Scenario 1: Progeny testing

A breeding program with claw health as the only breeding goal was assumed. A single claw disorder was used as an index trait. Bulls are selected based on phenotypic information on their progeny. Each bull is assumed to have 25 half-sib female offspring with phenotypes. Selection on dams is ignored. Generation interval is assumed to be 7 years for bulls and 5 years for dams. Results for selection response based on progeny testing are shown in Table 6.2. Selection response for claw disorders ranged from -0.02 (DS, SH, and SU) to -0.08 (IH) after ten years of selection.

6.4.2 Scenario 2: Genomic selection

Again, a breeding program with claw health as the only breeding goal was assumed. A single claw disorder was used as an index trait. Bulls are genotyped and selection is based on genomic information. The accuracies for GEBV were estimated in section 6.3.2, based on 70 genotyped bulls in the reference population. Generation interval is assumed to be 2 years for both bulls and dams. Results for selection response after ten years for different claw disorders are shown in Table 6.2. Selection response ranged from -0.03 (SH) to -0.12 (WLS). Selection response using genomic information (with 70 bulls in the reference population with DYD based on 25 daughters) is higher as compared to progeny testing (based on 25 daughters).

A response after ten years of selection of for example -0.06 can be achieved for infectious lesions using genomic selection. With an average claw disorder score of 0.238 for infectious lesions, this can be reduced to $0.238 - 0.06 = 0.178$ in ten years by approximation. However, it is not likely that a breeding program will focus solely on a specific claw disorder. Therefore, this can be seen as the maximum possible response. Due to genetic correlations different from unity with other traits in the

index of the breeding goal (and also with other claw disorders), selection response will be lower than indicated.

Table 6.2. Selection response (ΔG) for single trait selection after 10 years estimated by SelAction, based on progeny information on 25 daughters (P25) or genomic information (G).

Claw disorder	Abbreviation	ΔG / 10 years	
		P25	G
Double sole	DS	-0.02	-0.05
Infectious lesions	DER	-0.04	-0.06
Interdigital hyperplasia	IH	-0.08	-0.09
White line separation	WLS	-0.04	-0.12
Sole hemorrhage	SH	-0.02	-0.03
Sole ulcer	SU	-0.02	-0.05

6.4.3 Scenario 3: Correlated response

To investigate the correlated response of selection, a breeding program aimed at simultaneously improving milk production and claw health was assumed. The breeding goal (H) was: $H = v_1 A_{\text{milk production}} + v_2 A_{\text{claw disorders}}$. The index traits were sole ulcer (for claw disorders) and protein yield (kg) (for milk production) and these traits were included with different weights (v_1 and v_2 , resp.) in the breeding goal. Sole ulcer is used as an example, as this is an important claw disorder due to the high costs involved. The genetic correlation between 305d protein yield and sole ulcer was taken from Buch et al. (2011) who estimated a genetic correlation of 0.20. Heritability for protein yield was 0.27 and phenotypic variance was $1,500 \text{ kg}^2$ (Pritchard et al. 2012). In order to quantify how large the correlated genetic response is in claw disorders, a scenario was used with selection for milk production only, i.e. $v_1=1$ and $v_2=0$. In addition, scenarios were used with $v_1=0$ and $v_2=1$ (i.e. selection for claw disorders only) and weights different from 0 for both traits in the index. For claw disorders, a progeny group with 25 half-sib progeny was assumed and for milk production 100 half-sib progeny. Table 6.3 shows the (correlated) selection response, when selection is based on claw disorders only, milk production only, or on claw disorders and milk production together. Selection for increased milk production only, results in an increase of almost 23kg, but also an increase in claw disorder frequency of 0.015, after 10 years. Without selection for claw disorders, frequency will increase. When selection is only on reduced claw disorder frequency, a reduction of 0.025 after 10 years was estimated. In that scenario, milk production reduced with 9.35 kg after 10 years.

To ensure that a positive response is realized in milk production and claw disorders, both traits need to be in breeding goal and index. For that scenario, different weights in the breeding goal were used in order to obtain a response for claw disorders of at least 0 to avoid the negative correlated response. A weight of 1 is used for milk production. In order to use this weight, a weight of at least -371 is needed for claw disorders to obtain a response of at least 0. With a weight of -550 for claw disorders, an absolute decrease of 0.005 in claw disorder frequency is obtained. Reducing claw disorder incidence is feasible but comes at a cost of reduced production.

Table 6.3. Selection response when selection is based on sole ulcer only, protein yield only, or sole ulcer and protein yield combined with different weights. Results are given for 10 years of selection and estimated by SelAction, based on progeny information on 25 daughters for sole ulcer and 100 daughters for protein yield.

Breeding goal	Selection response after 10 years	
	Sole ulcer	Protein yield (kg)
Sole ulcer	-0.025	-9.35
Protein Yield	+0.015	+22.94
Sole ulcer + protein yield ¹	0.000	+21.02
Sole ulcer + protein yield ²	-0.005	+18.70

¹ a weight of 1 was used for protein yield and -371 for sole ulcer.

² a weight of 1 was used for protein yield and -550 for sole ulcer.

6.4.4 Conclusions

The genomic selection scenario had a lower accuracy but resulted in higher selection response compared to selection based on progeny information due to the shorter generation interval. Including more animals in the reference population will increase accuracy and thereby response to selection. Not including claw disorders at all, will lead to higher claw disorder frequencies over time. A selection response of -0.025 can be realized in a scheme that is focused on claw disorders only. Selection, however, is always on a combination of traits including milk production. Due to unfavorable genetic correlations with production traits in the breeding goal, selection response for claw disorders in real life will be lower and for some claw disorders keeping the incidence at the same level might be the most realistic scenario.

Text box 6.1. Input parameters SelAction

Simulated population: 20 bulls x 200 dams

- One-stage selection with discrete generations
- 0.5 male and 0.5 female offspring
- 10% selected proportion in bulls and 99.9% in dams
- Selection in bulls only

Trait	Heritability (h^2)	Accuracy of genomic selection ($r_{ih,GEBV}$)
Double Sole (DS)	0.02	0.31
Infectious lesions (DER)	0.04	0.35
Interdigital hyperplasia (IH)	0.14	0.45
Sole hemorrhage (SH)	0.02	0.29
Sole Ulcer (SU)	0.03	0.24
White line separation (WLS)	0.04	0.41
Protein Yield (PY)*	0.27	-

*estimated in Pritchard et al., 2012

Genomic information can be modelled as a separate trait (GEBV), with $h^2 = 0.99$; a genetic correlation between GEBV and claw disorder =

$$r_g = r_{ih,GEBV};$$

a phenotypic correlation between GEBV and claw disorder =

$$r_p = r_{ih,GEBV} * h_{claw\ disorder};$$

phenotypic variance of GEBV =

$$\sigma_p^2 = r_{ih,GEBV}^2 * \sigma_a^2_{claw\ disorder}.$$

Scenario 1: Breeding goal is improving claw health. Index trait is a single claw disorder. Information source is 25 progeny.

Scenario 2: Breeding goal is improving claw health. Index trait is a single claw disorder. Information source is genomic information.

Scenario 3: Breeding goal is improving milk production and claw health. Index trait is either protein yield, sole ulcer, or protein yield and sole ulcer combined. Information source is 100 progeny for protein yield and 25 progeny for sole ulcer. Genetic correlation between protein yield and sole ulcer is 0.20 (Buch et al., 2011).

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Summary

Summary

Claw disorders are important traits in dairy cattle breeding both from an economical and welfare point of view. Claw disorders have a multifactorial background and both environmental and genetic factors influence the development of claw disorders. Genetic selection can have a cumulative and permanent effect on claw disorder incidence. Aim of this thesis was to increase our understanding of the genetic background of claw disorders to enable selection for reduced claw disorder incidence.

In chapter 2 data was used from Holstein cows kept in dairy herds in France, mainly the Northeast region. Data was collected between 2007 and 2012. Fourteen different claw disorders were scored by professional claw trimmers from two trimming organizations. These claw disorders were: abscess, corkscrew claw, (inter-)digital dermatitis or heel erosion (DER), double sole (DS), hardship groove, interdigital hyperplasia (IH), interdigital phlegmon, sand crack, super-foul, sole hemorrhage (SH), sole injury, sole ulcer (SU), white line separation (WLS), and yellow discoloration of the sole. Individual claw disorder frequencies ranged from 0.1% to 23.8% and more than half of the trimmed cows had at least one claw disorder in at least one hind leg. Heritabilities were estimated for the six most common claw disorders (DER, DS, IH, SH, SU, and WLS), as other claw disorders were rarely recorded with frequencies below 3%. Heritabilities on the observed scale ranged from 0.02 to 0.14 (± 0.01). Repeatabilities ranged from 0.02 to 0.33 (± 0.01), indicating that repeated observations improve accuracy of estimated breeding values. Estimated genetic correlations between claw disorders were moderate to high between laminitis-related claw disorders (DS, SH, SU, WLS) and also between hygiene-related claw disorders (DER, IH). In our study it was also shown that we could not detect an effect of preselecting cows to be trimmed on claw disorder heritability. However, heritability tended to decrease when herds with a higher fraction of trimmed cows were selected for analyses. The need for trimming ("trimming status") was found to be heritable as well with a heritability of 0.09 (± 0.01) on the observed scale and was genetically correlated to claw disorders. Trimming status is a bulk trait containing information not only on claw disorders but also on excessive horn growth as almost half of all trimmed cows did not have a claw disorder. Trimming status, or an equivalent trait, is currently not considered in dairy cattle breeding. A high need for trimming the claws of cows is unfavorable and therefore trimming status is an interesting trait to include in the genetic evaluation.

Genome-wide association studies (GWAS) enable the detection of genetic variants associated with a particular trait or disease. Performing a GWAS might add to a better understanding of the development of claw disorders and the need for trimming, when the underlying genetic background can be identified. In chapter 3 a GWAS was performed for claw disorders and trimming status and validated in an independent data set. A single-nucleotide polymorphism (SNP) was considered significant when the false discovery rate was ≤ 0.05 and suggestive when the false discovery rate was ≤ 0.20 .

In total, 10 significant and 45 suggestive SNP were detected for claw disorders in a data set of 1,771 genotyped cows. All significant SNP were associated with sole ulcer and mainly located on chromosome 8. The suggestive SNP were associated with double sole, interdigital hyperplasia, sole ulcer, and a combination of laminitis-related claw disorders. Using a data set of 185 genotyped bulls, three of the suggestive SNP could be validated. One significant and one suggestive SNP on chromosome 15 were detected for trimming status. Previous studies have detected SNP associated with feet and leg conformation traits and were closely located to some of the SNP detected for claw disorders. Major genes associated with claw disorders or trimming status could not be identified and the significant and suggestive SNP were spread across the genome. This suggests that claw disorders are influenced by many genes each explaining a small proportion of the genetic variance. Therefore, selection for specific genes is not a feasible option, however, genomic selection can capture information of many genes with small effects and is a promising approach to reduce the incidence of claw disorders by selection.

Several traits, like milk production and fertility, are genetically not the same trait in different parities or lactation stages. Susceptibility to disease has been shown to be genetically different in environments with different herd disease prevalence. Limited knowledge is available on whether claw health traits (claw disorders and trimming status) are genetically the same trait in different parities, lactation stages, or in herds with low or high trait frequency. In order to improve genetic selection programs and data collection, it was investigated in chapter 4 whether claw health traits were genetically the same in first or later parities, during early or late lactation and in herds with a low or high trait frequency. Analyses showed that most claw health traits had similar heritabilities and were genetically the same in different parities, lactation stages and herds with different trait frequencies. Sole hemorrhage and infectious lesions were genetically different in first parity and later parities. Infectious lesions and white line separation were genetically different during peak or late lactation and sole ulcer was genetically different in herds with a low or high frequency of sole ulcer. In our analysis we did not find convincing

evidence (due to high standard errors) which supports the use of multiple trait models for the analysis of claw health traits, treating them as different traits in different parities, lactation stages, and herds with different claw disorder frequency.

Limited information is available on claw health in Montbeliarde cows. Furthermore, being the first to consider the trait trimming status, it is of interest to investigate this trait in another breed. In chapter 5, heritabilities and repeatabilities for claw disorders and trimming status were estimated. Also, genetic correlations between trimming status and claw disorders were estimated. The Montbeliarde cows were kept under similar conditions and trimmed by the same claw trimmers as the previously investigated Holstein population (chapter 2). Claw disorder frequency in Montbeliarde ranged from 9.4% to 41.1% and 73% of the cows had at least one claw disorder in at least one hind leg between 2007 and 2013. Heritability of claw disorders and trimming status were overall slightly lower as compared to the estimates in Holstein. Heritabilities ranged from 0.01 (± 0.01) to 0.09 (± 0.02). Heritability for trimming status was 0.06. The estimated heritability for trimming status in Montbeliarde was slightly lower than in Holstein. Genetic selection to improve claw health is feasible in Montbeliarde and this study confirms that trimming status is a heritable trait.

In the general discussion, chapter 6, results from previous chapters were put in broader perspective and three issues were discussed. The first part discusses several alternative methods to score claw health to improve heritability estimates and accuracy of estimating breeding values. Discussed were improved definition of claw disorder phenotypes and alternative ways of measuring claw disorders; pressure plates, infrared thermography, activity monitors, conformation scoring, and measuring the amount of horn trimmed. Convincing evidence that these methods can increase heritability or accuracy of breeding value estimation was not found. It was concluded that collecting direct scores for claw disorders is currently the best way to obtain information on claw disorders. In the second part, accuracy of progeny testing and genomic selection for claw disorders is compared based on theoretical predictions. Also, accuracy for genomic selection was estimated based on real data and compared to the theoretically expected accuracy. The accuracy of the EBV of a progeny tested bull with 25 daughters which are scored for claw disorders and assuming a claw disorder heritability of 0.05 is equal to 0.49. In order to obtain the same accuracy with genomic selection, a reference population consisting of 840 progeny tested bulls is needed where each bull has 25 daughters. Realized accuracies for claw disorders based on real data (0.24 – 0.45) were higher as compared to theoretically predicted accuracies (0.11 – 0.22). This is likely due to

genetic relationships between animals in the reference population and the validation population. Finally, a breeding program to reduce claw disorders was simulated in order to estimate selection response. It was shown that genomic selection (with 70 bulls in the reference population) results in a slightly higher selection response as compared to progeny testing when bulls had 25 daughters phenotyped for claw disorders. Selection solely for increased production without considering claw disorders in the breeding goal results in an increased incidence of claw disorders. A breeding goal consisting of two traits was simulated, including milk production and claw disorders. Protein yield was used as a trait for milk production and sole ulcer for claw disorders. Preventing an increase of claw disorder incidence comes at a cost of 1.92 kg protein after 10 years of selection (as compared to single-trait selection for production). However, selection for production without considering claw disorders, results in an absolute increase of claw disorders of roughly 2% in 10 years. Reducing the incidence of claw disorders is achievable with selection, but at a cost of a decrease in production.

Samenvatting

Samenvatting

Klauwaandoeningen zijn belangrijk in de fokkerij van melkvee, niet alleen vanuit economisch perspectief maar ook vanwege dierwelzijn. Klauwaandoeningen hebben een multifactoriële achtergrond en genetische factoren beïnvloeden de ontwikkeling van klauwaandoeningen. Genetische selectie kan een cumulatief en permanent effect hebben op de incidentie van klauwaandoeningen. Het doel van dit onderzoek was om meer inzicht te verkrijgen in de genetische achtergrond van klauwaandoeningen om selectie voor lagere incidentie van klauwaandoeningen mogelijk te maken.

In hoofdstuk 2 was data van Holstein koeien gebruikt die gehouden werden op bedrijven in Frankrijk, voornamelijk in het noordwesten. De data was verzameld tussen 2007 en 2012. Veertien verschillende klauwaandoeningen waren gescoord door professionele klauwbekappers afkomstig van twee bekaporganisaties. Deze klauwaandoeningen waren: (inter-)digitale dermatitis en/of stinkpoot (samen infectieuze aandoeningen; DER), dubbele zool (DS), horizontale groeven, verticale groeven, tyloom (IH), panaritium, tussenklauwontsteking, zoolbloeding (SH), zool verwonding, zoolzweer (SU), witte lijn aandoening (WLS) en gele verkleuring van de zool. Frequenties van individuele klauwaandoeningen varieerden van 0.1% tot 23.8% en meer dan de helft van de bekapte koeien had minimaal één klauwaandoening in ten minste één achterpoot. Erfelijkheidsgraden zijn geschat voor de zes meest voorkomende klauwaandoeningen (DER, DS, IH, SH, SU en WLS), aangezien andere klauwaandoeningen zelden werden waargenomen met frequenties onder de 3%. Erfelijkheidsgraden varieerden van 0.02 tot 0.14 (± 0.01). Herhaalbaarheden varieerden van 0.02 tot 0.33 (± 0.01), wat aangeeft dat herhaalde observaties de nauwkeurigheid van geschatte fokwaarden verbeterd. Geschatte genetische correlaties tussen klauwaandoeningen waren gemiddeld tot hoog voor laminitis-gerelateerde klauwaandoeningen (DS, SH, SU, WLS) en ook voor hygiëne-gerelateerde klauwaandoeningen (DER, IH). In deze studie heb ik ook laten zien dat een effect van het voorselecteren van koeien om te bekappen op de erfelijkheidsgraden niet gevonden werd, maar wanneer bedrijven werden geselecteerd waar een hoger percentage koeien bekap werd leek de erfelijkheidsgraad af te nemen. De behoefte om een koe te bekappen ("bekapstatus") was ook erfelijk met een erfelijkheidsgraad van 0.09 (± 0.01) en was genetisch gecorreleerd met klauwaandoeningen. Bekapstatus is een verzamelkenmerk dat niet alleen informatie bevat over klauwaandoeningen maar bijvoorbeeld ook over excessieve hoorn groei aangezien bijna de helft van de bekapte koeien geen klauwaandoening had, maar toch bekap werd. Bekapstatus,

of een vergelijkbaar kenmerk, wordt momenteel niet meegenomen in de melkvee fokkerij. Een hoge bekap behoefte is niet gewenst en daarom is bekapstatus een interessant kenmerk om mee te nemen in de genetische evaluatie.

Associatie studies met markers verspreid over het hele genoom (GWAS) maken het mogelijk om genetische markers te detecteren die geassocieerd zijn met een kenmerk of ziekte. Het uitvoeren van een GWAS kan bijdragen aan een beter begrip van de ontwikkeling van klauwaandoeningen en de bekap behoefte, wanneer de onderliggende genetische achtergrond geïdentificeerd kan worden. In hoofdstuk 3 is een GWAS uitgevoerd voor klauwaandoeningen en bekapstatus en de GWAS is gevalideerd in een onafhankelijke dataset. Een marker (SNP) werd significant bevonden bij een kans van maximaal 5% op een foutieve detectie en suggestief bij maximaal 20%. In totaal werden 10 significante en 45 suggestieve SNP gevonden voor klauwaandoeningen in een dataset van 1.771 gegenotypeerde koeien. Alle significante SNP waren geassocieerd met zoolweer en lagen voornamelijk op chromosoom 8. De suggestieve SNP waren geassocieerd met dubbele zool, tyloom, zoolweer en een combinatie van laminitis-gerelateerde klauwaandoeningen. Met een dataset van 185 gegenotypeerde stieren konden 3 suggestieve SNP worden gevalideerd. Een significante en een suggestieve SNP op chromosoom 15 werden gedetecteerd voor bekapstatus. Voorgaande studies hebben SNP gedetecteerd die geassocieerd waren met klauw en been conformatie kenmerken en lagen dichtbij een aantal SNP gedetecteerd voor klauwproblemen. Genen met grote invloed op klauwaandoeningen en bekapstatus werden niet gevonden, daarnaast lagen de significante en suggestieve SNP verspreid over het genoom. Dat suggereert dat klauwaandoeningen beïnvloed worden door veel genen die ieder een klein deel van de genetische variantie verklaren. Daarom is selectie van specifieke genen niet mogelijk, maar genomische selectie kan informatie oppikken van vele genen ieder met een klein effect en is een veelbelovende selectie methode om de incidentie van klauwaandoeningen te reduceren.

Voor een aantal kenmerken als melkproductie en fertiliteit, zijn er verschillen in genetische parameters tussen pariteiten en lactatie stadia. Gevoeligheid voor ziekte is genetisch verschillend onder omstandigheden met verschillende prevalentie van ziektes op het bedrijf. Beperkte kennis is beschikbaar of klauwgezondheid (klauwaandoeningen en bekapstatus) genetisch hetzelfde is in verschillende pariteiten, lactatie stadia of op bedrijven met lage of hoge frequentie van het kenmerk. Om genetische selectie programma's en het verzamelen van data te verbeteren, was in hoofdstuk 4 onderzocht of klauw gezondheid genetisch hetzelfde is in eerste of latere pariteit, tijdens het begin of later in lactatie en op

bedrijven met een lage of hoge frequentie van de klauwaandoeningen. Uit analyses blijkt dat de meeste klauwgezondheidskenmerken vergelijkbare erfelijkheidsgraden hadden en genetisch hetzelfde kenmerk waren in verschillende pariteiten, lactatie stadia en op bedrijven met verschillende frequenties van klauwaandoeningen. Zoolzweer en infectieuze aandoeningen waren genetisch verschillend in eerste en latere pariteit. Infectieuze aandoeningen en witte lijn aandoening waren genetisch verschillend tijdens het begin en later in lactatie en zoolzweer was genetisch verschillend op bedrijven met een hoge of lage zoolzweer frequentie. Overtuigend bewijs werd niet gevonden (vanwege hoge standaardfouten) voor het gebruik van een multi-kenmerk model voor klauwgezondheidskenmerken, waarbij ze als verschillende kenmerken worden behandeld voor verschillende pariteiten, lactatiestadia of bedrijven met verschillende frequenties van klauwaandoeningen. Beperkte informatie is beschikbaar over klauwgezondheid in Montbeliarde koeien. Omdat dit de eerste studie is die bekapstatus overweegt, is het van belang om dit kenmerk te onderzoeken in een ander ras. In hoofdstuk 5 werden erfelijkheidsgraden en herhaalbaarheden geschat voor klauw aandoeningen en bekapstatus in Montbeliarde. Daarnaast werden genetische correlaties tussen klauwaandoeningen en bekapstatus geschat. De Montbeliarde koeien werden gehouden onder vergelijkbare omstandigheden en werden bekapst door dezelfde bekappers als de eerder onderzochte Holstein koeien (hoofdstuk 2). Frequenties van klauwaandoeningen in Montbeliarde varieerden van 9.4% tot 41.1% en 73% van de koeien had minimaal één klauwaandoening in ten minste één achterpoot tussen 2007 en 2013. De erfelijkheidsgraden voor klauwaandoeningen en bekapstatus waren over het algemeen iets lager dan de schattingen in Holstein. Erfelijkheidsgraden varieerden van 0.01 (± 0.01) tot 0.09 (± 0.02). De erfelijkheidsgraad voor bekapstatus was 0.06. De geschatte erfelijkheidsgraad voor bekapstatus in Montbeliarde was iets lager dan in Holstein. Genetische selectie voor het verbeteren van klauwgezondheid is mogelijk in Montbeliarde en de resultaten bevestigen de erfelijkheid van bekapstatus.

In de algemene discussie, hoofdstuk 6, werden de resultaten van de eerdere hoofdstukken in groter perspectief geplaatst en drie onderwerpen werden nader bekeken. In het eerste deel werden een aantal alternatieve methoden om klauwaandoeningen te scoren, ter verbetering van de erfelijkheidsgraden en nauwkeurigheid van geschatte fokwaarden, bediscussieerd. Het verbeteren van het fenotyperen van klauwaandoeningen en alternatieve methoden om klauwaandoeningen te meten werden beschreven, zoals drukplaten, infrarood thermografie, activiteitsmeters, het scoren van conformatie en het meten van de hoeveelheid bekapte hoorn. Overtuigend bewijs dat deze methoden de

erfelijkheidsgraden of de nauwkeurigheid van het schatten van fokwaarden kunnen verbeteren was niet gevonden. Het scoren van klauwaandoeningen zoals het nu gebeurt, is de beste methode op dit moment om data te verzamelen over klauwaandoeningen.

In het tweede deel werd een vergelijking gemaakt tussen de nauwkeurigheid van selectie gebaseerd op een nakomelingen test en selectie gebaseerd op genomische informatie, voorspeld met theoretische modellen. Daarnaast werd de nauwkeurigheid voor selectie gebaseerd op genomische informatie berekend met echte data en vergeleken met de theoretische verwachting. De nauwkeurigheid van een fokwaarde voor een stier gebaseerd op 25 dochters met scores voor klauwaandoeningen met een erfelijkheidsgraad van 0.05 is gelijk aan 0.49. Om dezelfde nauwkeurigheid te krijgen bij selectie gebaseerd op genomische informatie, is een referentie populatie nodig van 840 stieren met ieder 25 dochters met klauw data. Gerealiseerde nauwkeurigheden voor klauwaandoeningen gebaseerd op echte data (0.24 – 0.45) waren hoger dan verwacht op basis van theoretische voorspellingen (0.11 – 0.22). Dat komt waarschijnlijk door verwantschap tussen dieren in de referentie en validatie populatie.

In het derde deel werd een fokprogramma om klauwaandoeningen te reduceren gesimuleerd om de selectie response te schatten. Het gebruiken van genomische informatie (met minimaal 70 stieren in de referentiepopulatie) resulteerde in een iets hogere selectie response als de nakomelingen test bij stieren met minimaal 25 dochters met klauw data. Selectie uitsluitend gebaseerd op productie eigenschappen zonder klauwaandoeningen mee te nemen, resulteerde in verhoogde incidentie van klauwaandoeningen. Een fokdoel met twee kenmerken, namelijk melk productie en klauwaandoeningen, werd gesimuleerd. Eiwit productie was gebruikt als eigenschap voor melk productie en zoolzweer voor klauwaandoeningen. Om een stijging in incidentie van klauwaandoeningen te voorkomen, betekent dat een verlies van 1.92 kg eiwit na tien jaar van selectie (vergeleken met selectie enkel op productie). Aan de andere kant resulteert selectie gebaseerd op productie alleen, zonder klauwaandoeningen in overweging te nemen, in een absolute stijging van ongeveer 2% incidentie van klauwaandoeningen na tien jaar. Het terugdringen van de incidentie van klauwaandoeningen is mogelijk met behulp van selectie maar gaat ten koste van productie.

Curriculum Vitae

About the author

Dianne van der Spek was born on the 2nd of January 1984 in Moerkapelle, the Netherlands, where she was also raised. In 2002 she obtained her high school diploma from Driestar College, Gouda. In the same year she started studying Psychology at the Vrije Universiteit of Amsterdam where she obtained a propaedeutic diploma. After studying Veterinary Science at the University of Gent (Belgium) for two years, she had discovered Wageningen University and became very much interested in studying Animal Breeding and Genetics. In 2005 she started her BSc studies in Animal Science at Wageningen University, followed by a double-degree European Master of Science program in Animal Breeding and Genetics (EMABG). Dianne spent her first year of EMABG at Wageningen University and the second year at the Swedish University of Agriculture (SLU) in Uppsala, Sweden. At both universities she wrote a major MSc thesis. For the first MSc thesis, she collected data on feather pecking behaviour in laying hens and calculated expected and realized selection response to reduce feather pecking in laying hens. For the second MSc thesis, she extracted DNA from canine blood samples which was consequently genotyped and she analysed the genotypes for associations of MHC class II genes with three immunological disorders in different dog breeds. After finishing her master studies in 2010, she worked as a teaching assistant at the Animal Breeding and Genomics Centre of Wageningen University. She rewrote a chapter of the study material of the BSc course Applied Animal Biology. She also set up a new electronic practical about DNA and an electronic exam. In 2011 she started her PhD research in the same group, as a collaboration with Gènes Diffusion in France. The results of this research are described in this thesis. Currently, Dianne is working as a geneticist at Topigs Norsvin Research Center in Beuningen, the Netherlands.

Over de auteur

Dianne van der Spek is geboren op 2 januari 1984 in Moerkapelle, Nederland, waar ze ook is opgegroeid. Ze behaalde haar gymnasium diploma in 2002 aan het Driestar College te Gouda. In datzelfde jaar begon ze aan de studie Psychologie aan de Vrije Universiteit van Amsterdam waar ze haar propedeutisch diploma behaalde. Na twee jaar Diergeneeskunde gestudeerd te hebben aan Universiteit Gent (België), ontdekte ze Wageningen Universiteit en raakte ze geïnteresseerd in de richting Veefokkerij en Genetica. In 2005 begon ze dan ook aan de bachelor opleiding dierwetenschappen aan Wageningen Universiteit, aansluitend gevolgd door een Europese double-degree master in Veefokkerij en Genetica (EMABG). Dianne bracht haar eerste EMABG jaar door aan Wageningen Universiteit en het tweede jaar aan de Zweedse Landbouw Universiteit (SLU) in Uppsala, Zweden. Bij beide universiteiten schreef ze een major thesis. Voor haar eerste MSc thesis verzamelde ze data op een leghennenbedrijf over verenpikgedrag bij leghennen. Ze berekende verwachte en gerealiseerde selectie respons om verenpikken te reduceren in leghennen. Voor haar tweede MSc thesis isoleerde ze DNA uit bloed monsters van honden wat vervolgens werd gegenotypeerd en ze analyseerde de genotypes op associaties van MHC type II genen met immunologische ziektes bij verschillende hondenrassen. Na het voltooien van haar master opleiding in 2010, werkte ze als onderwijs assistente bij de vakgroep Veefokkerij en Genetica aan Wageningen Universiteit. Ze herschreef een hoofdstuk van het studiemateriaal voor het bachelor vak Toegepaste Dierbiologie. Ze heeft ook een nieuw elektronisch practicum over DNA opgezet en een elektronisch examen voor het vak. In 2011 begon ze haar AIO onderzoek bij dezelfde vakgroep, als een samenwerking met Gènes Diffusion in Frankrijk. De resultaten van dit onderzoek zijn beschreven in deze thesis. Momenteel is Dianne werkzaam bij Topigs Norsvin Research Center in Beuningen, Nederland.

Publications

Peer reviewed publications

Van der Spek, D., J.A.M. Van Arendonk, A.A.A. Vallée, and H. Bovenhuis. 2013. Genetic parameters for claw disorders and the effect of preselecting cows for trimming. *Journal of Dairy Science* 96:6070-6078.

Van der Spek, D., J.A.M. van Arendonk, and H. Bovenhuis. 2015. Genome-wide association study for claw disorders and trimming status in dairy cattle. *Journal of Dairy Science* 98: 1286-1295.

Van der Spek, D., J. A. M. van Arendonk, and H. Bovenhuis. 2015. Genetic relationships between claw health traits of dairy cows in different parities, lactation stages, and herds with different claw disorder frequencies. *Journal of Dairy Science*, *in press*.

Manuscript in preparation

Van der Spek, D., M. Theloosen, J.A.M. Van Arendonk, and H. Bovenhuis. Genetic background of claw disorders and trimming status in Montbeliarde cows.

Conference proceedings

Van der Spek, D., J.A.M. van Arendonk, A.A.A. Vallée, and H. Bovenhuis. Genetic parameters of hoof lesions in French Holstein dairy cattle. 63rd annual meeting of the European Association for Animal Production. 27-31 August 2012. Bratislava, Slovakia.

Van der Spek, D., J.A.M. Van Arendonk, A.A.A. Vallée, and H. Bovenhuis. Claw disorder genetics and the effect of preselecting cows for trimming. 9th International Conference on Lameness in Ruminants. 11-14 August 2013. Bristol, United Kingdom.

Van der Spek, D., J.A.M. Van Arendonk, and H. Bovenhuis. Laminitis-related claw disorders in dairy cattle: A genome-wide association study. 10th World Congress on Genetics Applied to Livestock Production. 17-22 August 2014.

Popular publication

Van der Spek, D. Interview. By I. Stienezen. 2015. Fokken op productie vermindert klauwgezondheid. Veeteelt 10, pages 38-39.

Training and education

Training and supervision plan (TSP)



The basic package (3 ECTS)

WIAS introduction course	2011
Ethics and philosophy in life sciences	2013

Scientific exposure (13 ECTS)

International conferences

62 nd EAAP, Stavanger, Norway	2011
63 rd EAAP, Bratislava, Slovakia	2012
International conference on lameness in ruminants, Bristol, United Kingdom	2013
10 th WCGALP, Vancouver, Canada	2014

Seminars and workshops

Friends or fiends? Consequences of social interactions for artificial breeding programs and evolution in natural populations	2009
Fokkerij en Genetica connectie dagen	2010,2012,2014
Genomics and animal breeding	2011
WIAS science day	2012-2014
Genomic selection for novel traits	2014

Presentations

Oral presentation, EAAP, Bratislava, Slovakia	2012
Oral presentation, ICLR, Bristol, United Kingdom	2013
Poster presentation, WIAS science day, Wageningen	2013
Oral presentation, WCGALP, Vancouver, Canada	2014

In-depth studies (8 ECTS)

Disciplinary and interdisciplinary courses

Genomic selection in livestock	2011
Identity by descent approaches to genomic analyses of genetic traits	2012
Advanced methods and algorithms in animal breeding with focus on genomic selection	2012
Advanced statistical and genetic analysis of complex data using ASReml4	2014
Introduction to theory and implementation of genomic selection	2014

PhD students' discussion groups

Quantitative genetics discussion group	2011-2015
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Professional skills support courses (4 ECTS)

Techniques for writing and presenting a scientific paper	2012
Supervising thesis students	2012
Effective behaviour in professional surroundings	2013
Career perspectives	2014

Research skills training (7 ECTS)

Preparing own PhD research proposal	2011
Introduction to R for statistical analyses	2012
Getting started in ASReml	2012

Didactic skills training (16 ECTS)

Lecturing

BSc course 'Toegepaste Dierbiologie', Wageningen	2011
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Supervising practicals and excursions

BSc course 'Toegepaste Dierbiologie', Wageningen	2011-2014
BSc course 'Animal Breeding and Genetics', Wageningen	2010-2011
Review RMC proposals, Wageningen	2012
MSc course 'Genetic improvement of livestock', Wageningen	2013

Training and education

Supervising theses

MSc thesis, Animal Breeding and Genetics 2014

Preparing course material

BSc course 'Toegepaste Dierbiologie', Wageningen 2010

Management skills training (1 ECTS)

Organizing Quantitative Genetics Discussion Group, Wageningen 2013-2014

Training and education total 53 ECTS

Dankwoord

Dankwoord

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Amélie, thanks for your help with the data, your suggestions, involvement and being a translator whenever needed. Gaël, Weijia, Christophe, Sophie and all other colleagues from Gènes Diffusion; thanks for your help with the blood samples, the genotyping, the discussions, the lunches together, your friendliness, and your effort to speak English with me. Very much appreciated! Pascal and Perrine, your explanations about the data and your valuable time to answer my questions made my start so much easier! I would also like to thank the claw trimmers for collecting the data and showing me how you trim the cows and how you score the claw disorders. Your enthusiasm about explaining your work was contagious!

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Fellow PhD students at the Animal Breeding and Genomics Centre, thank you for the discussions, the coffee/tea breaks, sharing an office with me in the old and new Zodiac and in Radix, the pub quizzes (Yvonne, thanks for inviting me even after I left the office and even if I don't know many of the answers ☺), dinners together, and everything else that made PhD life just that bit easier or more fun! Alex, bedankt voor je hulp op ICT gebied, je betrokkenheid en support. Sommige problemen blijken onoplosbaar (zoals een externe schijf die crasht), maar je deed er alles aan om het voor mij makkelijker te maken, super! Dames van het secretariaat, Ada en Lisette, bedankt voor jullie snelle en effectieve hulp wanneer nodig. Ilse, bedankt voor je hulp bij het nadenken over de stellingen. Ik vond het zelf maar lastig, maar dankzij jou werd het makkelijker om na te denken over geschikte onderwerpen. Marco, dankzij jou heb ik ook nog een beetje ervaring kunnen opdoen in de 'plantenwereld'. Al was de timing niet ideaal zo tijdens het schrijven van mijn algemene discussie, vond ik het toch heel interessant om in de pilot dataset mooie associaties te vinden met GWAS voor bijvoorbeeld zuurgraad in appel (de uitdrukking 'door de zure appel heen bijten' komt hier letterlijk en figuurlijk van pas ☺).

Susan, Sandrine, thank you for sitting next to me on this important day! Susan, gelukkig is het goed gekomen met de kopjes thee waar je over schreef in je eigen proefschrift ☺ en hopelijk volgen er nog veel meer. Bedankt voor alle gezelligheid tijdens en na je promotie traject en dat ik altijd bij je terecht kan voor een wetenschappelijke vraag, luisterend oor en een beetje afleiding (bijvoorbeeld met zelfgebakken taart). Wie had destijds gedacht dat we nu weer een kantoor zouden delen...

Sandrine, never a dull moment when you are around! Thanks for the discussions and fun times! Soon you will finish too (yes, you will), and wherever you will end up, I hope we will stay in touch!

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Liefs, Dianne

Colophon

Colophon

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