Genetic parameters and genome wide association study of individual hoof lesions in Canadian Holsteins using different contemporary groups

F. Malchiodi[*], A. Koeck[*], A.M. Christen[¥], F. S. Schenkel[*], D. F. Kelton‡, and F. Miglior[*†]

[*]Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, Ontario

[¥]Valacta, Sainte-Anne-de-Bellevue, Quebec

‡Department of Population Medicine, Ontario Veterinary College, University of Guelph

†Canadian Dairy Network, Guelph, Ontario

INTRODUCTION

Different studies performed in Europe and North America reported a high prevalence of hoof lesions in dairy herds, with 40 to 70% of cows suffering from at least one type of hoof lesion (Manske et al., 2002; Bunch et al., 2011). A hoof lesion represents a welfare problem (Bruijnis et al. 2012), as it is a painful condition for the cow, but it also poses an economical loss for farmers, due to the costs associated to treating the lesion, as well as to decreased cow performance. The presence of hoof lesions has been reported to affect milk production (Amory et al. 2007) and to be associated with a lower reproductive performance of cows (Hultgren et al., 2004). Therefore, it is important to reduce the incidence of hoof lesions, which can be achieved both by improving management practices, and through genetic selection. A low correlation between type classification traits and hoof lesion has been reported (Van der Linde et al., 2010; Chapinal et al., 2013), so that indirect selection for hoof health using feet and leg conformation traits has not been very efficient. This underscores the necessity to have a standardized system for data collection on hoof lesions, and their use for direct genetic selection. Previous studies on Canadian Holstein cows showed how hoof lesion data collected by hoof trimmers have the potential to be used for genetic evaluation (Chapinal et al., 2013). The reason for which cows have been examined is not usually reported, however, it should be also taken into consideration. The selection process in some cases may not be completely random, since cows that showed hoof problems are more likely to be preferentially sent for examination. This could result in erroneous estimations of the prevalence of hoof lesions in the farm, and also of the EBV of the sires. However, Van der Spek et al. (2013) reported that having trimming records on a selected sample of cows does not have a severe effect on the estimated heritabilities.

The objective of this study was to estimate genetic parameters for hoof lesions and to identify chromosome regions associated with hoof lesions in Canadian Holsteins, performing an alternative

analysis in order to consider all cows in the herd during the period of the hoof trimming sessions, also those that were not examined by the trimmer over the entire lactation.

MATERIAL AND METHODS

Data

Hoof lesions were recorded in 365 herds located in Alberta (from June 2009 to November 2012), British Columbia (from October 2010 to March 2012), Ontario (from August 2011 to July 2012), and Quebec (from January 2012 to July 2012). Data were recorded by 26 trimmers, trained to use a rugged touch-screen computerized lesion recording system (Hoof Supervisor, Dresser, WI), based on lesion descriptions proposed by the International Lameness Committee, a global collaboration of researchers, veterinarians, academics, and hoof-trimming professionals. Hoof lesions included in the analysis were digital dermatitis, interdigital dermatitis, interdigital hyperplasia, sole hemorrhage, sole ulcer, toe ulcer, and white line lesion. Due to low frequency corkscrew claw, heel erosion, foot rot, axial fissure, vertical fissure, horizontal fissure, thin sole and unidentified lesions were combined into a group denominated "other lesions". Finally, a variable was created, that included the absence or the presence of any of the hoof lesions previously described. All the traits were coded as binary variables (0; 1), where 1 was assigned to the presence of a lesion. Digital dermatitis, interdigital dermatitis, interdigital hyperplasia, sole hemorrhage, sole ulcer, toe ulcer, and white line lesion were also considered as categorical variables, using a severity score from 0 to 3. Trimming sessions recorded after 500 d postpartum were not used in the analysis. A minimum of 10 records per hoof trimming session per herd was required in order to include data for any particular day in the analysis. The majority of the cows (65%) were only hoof trimmed once during the same lactation, therefore, only the first hoof-trimming session was included in the analyses, as previously described by Buch et al. (2011). Finally, two different contemporary groups were created. The first group of cows (Group 1) included only data from cows that had been visited at least one time by the trimmer during the course of lactation. In a second group (Group 2), all the cows that were in the herds during the trimming period were included in the analyses, also cows that did not have any hoof data during the lactations. In such cases, the trimming session date was replaced with the first trimming session available in that herd during the lactation, and 0 was assigned to all hoof traits for that trimming session. The final data set consisted of 75,559 hoof-trimming records from 53,654 cows, when only trimmed cows where considered, and 104,446 hoof-trimming records from 70,394 cows, in the contemporary group. The final pedigree files for the first

and the second dataset contained 196,879 animals and 230,267 animals, respectively, and included 7 previous generations.

Model

Data were analyzed with a linear animal model, using the average information-restricted maximum likelihood (AI-REML) procedure, in the derivative-free approach to multivariate analysis (DMU) package (Madsen and Jensen, 2008). The following linear animal model was applied to all lesion traits:

$$Y_{ijklmnop} = \mu + HD_i + TRIMMER_j + PARITY_k + STAGE_l + a_m + pe_n + e_{ijklmnop},$$

where $Y_{ijklmno}$ is the observation for one of the lesion traits, μ is the overall mean, $HERD_l$ is the fixed effect of herd-date of hoof trimming (l = 1 to 3,086), $TRIMMER_k$ is the fixed effect of hoof trimmer (k = 1 to 26), $PARITY_k$ is the fixed effect of parity at trimming (k = 1 to 7 and later), $STAGE_l$ is the fixed effect of stage of lactation at trimming (l = 1 to 16; class1: 0 - 30 d, class2 = 31 - 60 d, …, class15: 421 - 450 d, and class16: 451 - 500 d after calving), a_m is the random additive genetic animal effect (m = 1 to 196,879, for Group 1; or 1 to 230,367, for Group 2), pe_n is the random permanent environmental effect (n = 1 to 53,654, for Group 1; or 1 to 70,394, for Group 2), and $e_{ijklmnop}$ is the random error term. Because heritability estimates are frequency-dependent when applying linear models to binary data (Gianola, 1982), possible differences between the two groups could be due by the difference in the prevalence of the lesions. In order to account for this, the method of Dempster and Lerner (1950) was used to transform heritabilities to the underlying scale (h^2_u).

GWAS Analyses

Estimated breeding values of sires with genotypes were considered for further analyses (n = 5,064). Sires were genotyped with 50k SNP panel and their genotypes were imputed to HD (777k) using FImpute software. A genome-wide association study was carried out using a single SNP regression method, which included traditional relationships to account for family structure.

RESULTS AND DISCUSSION

Prevalence of Hoof Lesions

The prevalence of hoof lesions across parity is reported in Tables 1 and 2. As expected, the prevalence of hoof lesions decreased when the second contemporary group was considered. The general prevalence of hoof lesions was similar to that reported by Amory et al. (2008), in which all the trimming sessions, and not only the first session for lactation, were considered. Generally, the

prevalence of digital dermatitis and interdigital dermatitis decreased across parity number, whereas the prevalence of sole hemorrhage, sole ulcer, toe ulcer, and white line lesions increased. These results are in agreement with results previously reported by Chapinal et al. (2013), in which an overall increase of horn lesions and a decrease of infection lesions across parity was shown. The differences in the patterns of change of these two types of lesions could be partially explained by the presence of irreversible hoof lesions, so that the prevalence increases with parity (Koening et al., 2005). On the other hand, the increase in local immunity with age could partially explain the decrease of infectious lesions (Chapinal et al., 2013). Importantly, it should be noted that cows with a predisposition to hoof problems may also suffer from other immune system-associated disorders, so that these cows can potentially be culled early from the herd, affecting their contribution to the data of prevalence of hoof problems as parity increases.

Heritabilities and Genetic Correlations of Hoof Lesions

When only hoof trimmed cows were included in the analysis, estimated heritability of foot lesions ranged from 0.006 to 0.067 (Table 3). Similar heritabilities for hoof lesions have been previously reported (Van der Linde et al., 2010; Chapinal et al., 2013). Chapinal et al. (2013) reported a heritability of 0.076 for infectious lesions. In the current study, digital dermatitis and interdigital dermatitis were considered as separate traits, and their heritabilities were 0.067 and 0.015, respectively, possibly affected by the large difference in prevalence between these two infectious lesions. Moreover, considering the hereditability on the underlying scale, digital dermatitis and interdigital dermatitis shown similar heritability coefficients of 0.14 and 0.13, respectively. Similarly, horn lesions were previously reported to have an estimated heritability of 0.028, ranging in the current study from 0.006 to 0.038 for different hoof lesion types. Koenig et al. (2005) obtained similar results for digital dermatitis, using a logistic approach, and estimated a heritability of 0.073. In the same study, slightly higher heritabilities were shown for sole ulcer and interdigital hyperplasia (0.086 and 0.104, respectively). The estimated heritability was slightly lower when contemporary group was included. However, when heritability on the underlying scale was considered, this difference was reduced, indicating that the aforementioned differences were probably mostly related to a lower frequency of hoof lesions in Group2. In agreement with findings reported by Van Der Spek et al. (2013), these results suggest that the pre-selection process of cows for trimming does not have a severe effect on the estimated heritabilities. Finally, the heritability on the underlying scale can help with comparisons of the coefficients estimated between groups with different frequency, but it may overestimate the

estimated heritability. Other approaches, such as the use of a threshold model, could be considered in order to better compare the use of different contemporary groups.

Genetic Correlation of Hoof Lesions

Genetic correlations between single hoof lesions were very similar among the two contemporary groups (Table4 and Table5). Infection lesions showed moderate genetic correlations with interdigital hyperplasia. Similarly, Van Der Spek et al. (2013) reported a correlation of 0.66 (0.08) between interdigital hyperplasia and dermatitis-erosion, which is a group that combined digital dermatitis, interdigital dermatitis and heel horn erosion. In the same study, zero genetic correlations were reported between dermatitis-erosion and any of the horn lesions. However, in our study low negative correlations were found between infection lesions (digital and interdigital dermatitis) and white line lesion. Among horn lesions, moderate to high genetic correlations were found. The higher correlation (0.83) was found between sole ulcer and sole hemorrhage. Further analyses will be performed in order to estimate the genetic correlation between single hoof lesions and conformation traits, such as foot angle, heel depth, bone quality, rear leg side view, and rear leg rear view, and locomotion.

Heritabilities of Hoof Lesions Using a Severity Score System

When the lesions were considered as a categorical variable, using a severity score from 0 to 3, the estimated heritability ranged from 0.005 to 0.052 (Table 6). The results were very similar to those estimated considering the lesions as binary traits (0; 1). The estimated breeding values between these two sets of variables showed correlations ranging from 0.87 to 0.97, and between 0.94 and 0.96 when only the EBVs of sires with at least 20 daughters were considered. The reliability of EBV was slightly higher when severity was analyzed. Because the previous analyses did not show a difference in the estimations between the two groups, only results for Group2 have been reported for this analysis.

GWAS for Digital Dermatitis, Interdigital Hyperplasia, and Sole Ulcer

Manhattan plots showing markers and chromosomal regions associated with digital dermatitis, interdigital hyperplasia, and sole ulcer are reported in Figure 1, 2, and 3, respectively. The results reported were calculated from the EBVs estimated from the analyses of Group 2 in which the traits were coded as binary variables (0; 1) and with reliability higher than 30. For digital dermatitis, a total of 720 SNP were significant at a 5% genome-wise FDR and significant peaks were detected on

chromosomes 1, 5, 8, 14 and 26, suggesting the presence of candidate genes in these regions. For interdigital hyperplasia 213 SNP were significant at a 5% genome-wise FDR, and significant peaks were detected only on chromosomes 5 and 22. However, these results could be due to a lower prevalence of this disease that resulted in smaller reliability of the EBV. Finally, for sole ulcer, a total of 712 SNP were significant at a 5% genome-wise FDR, and peaks were observed on chromosomes 4, 7, 10, 18, and 26. Further analyses on the detected areas on different chromosomes are necessary in order to establish whether this SNPs is harbored within the regions of known genes, allowing a more comprehensive understanding of the mechanisms involved in these different lesions.

CONCLUSIONS

The standardization of hoof lesion data collection is key to study this type of trait. The inclusion of non -trimmed cows in the data set seems not to influence the estimation of heritability for hoof lesions, but could help in avoiding the overestimation of the prevalence of hoof lesions in dairy herds. Further studies should be performed in order to estimate the genetic variation for hoof lesions in the Canadian Holstein population, and therefore, to explore the possibility for improvement through direct selection in the long term.

ACKNOWLEDGMENTS

The authors are grateful to the hoof trimmers who participated in this study. This research was supported by a contribution from the Dairy Research Cluster Initiative (Dairy Farmers of Canada, Agriculture and Agri-Food Canada, the Canadian Dairy Network and the Canadian Dairy Commission),Alberta Milk (Edmonton, AB, Canada) and Ontario Genomics Institute.

REFERENCES

Amory, J. R., Barker, Z. E., Wright, J. L., Mason, S. A., Blowey, R. W., and Green, L. E. 2008. Associations between sole ulcer, white line disease and digital dermatitis and the milk yield of 1824 dairy cows on 30 dairy cow farms in England and Wales from February 2003–November 2004. Prev. Vet. Med.: 83: 381-391.

Buch, L. H., Sørensen, A. C., Lassen, J., Berg, P., Eriksson, J. Å., Jakobsen, J. H., and Sørensen, M. K. 2011. Hygiene-related and feed-related hoof diseases show different patterns of genetic correlations to clinical mastitis and female fertility. J. Dairy Sci. : 94: 1540-1551.

Chapinal, N., Koeck, A., Sewalem, A., Kelton, D. F., Mason, S., Cramer, G., and Miglior, F. 2013. Genetic parameters for hoof lesions and their relationship with feet and leg traits in Canadian Holstein cows. J. Dairy Sci. : 96: 2596-2604.

Gianola, D. 1982. Theory and analysis of threshold characters. J. Anim. Sci. 54:1079–1096.

Hultgren, J., Manske, T., and Bergsten, C. 2004. Associations of sole ulcer at claw trimming with reproductive performance, udder health, milk yield, and culling in Swedish dairy cattle. Prev. Vet. Med. : 62: 233-251.

Koenig, S., A. R. Sharifi, H. Wentrot, D. Landmann, M. Eise, and H. Simianer. 2005. Genetic parameters of claw and foot disorders estimated with logistic models. J. Dairy Sci. 88:3316–3325.

Madsen, P., and J. Jensen. 2008. An User's Guide to DMU: A Package for Analyzing Multivariate Mixed Models. Version 6, release 4.7. Danish Institute of Agricultural Sciences, Tjele, Denmark.

Manske, T., Hultgren, J., and Bergsten, C. 2002. Prevalence and interrelationships of hoof lesions and lameness in Swedish dairy cows Prev. Vet. Med.: 54: 247-263.

Van der Linde, C., De Jong, G., Koenen, E. P. C., and Eding, H. (2010). Claw health index for Dutch dairy cattle based on claw trimming and conformation data. J. Dairy Sci. : 93: 4883-4891.

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. J. Dairy Sci. 96:6070–6078.

Table 1. Percentage of hoof trimming records with hoof lesions including only the first trimming session of the lactation from cows that has been visited at list one time by the trimmer during the lactation (Group 1), and stratified by parity

	All	Parity						
		1	2	3	4	5	6	≥ 7
Records (n)	73,559	26,989	20,726	12,916	7,202	3,458	1,523	745
Digital dermatitis (DD)	20.0	21.7	23.1	18.6	14.3	11.6	10.5	8.7
Interdigital dermatitis (ID)	1.9	1.7	2.1	2.2	1.9	2.0	1.0	1.2
Interdigital hyperplasia (IH)	2.1	1.2	2.4	2.8	2.7	2.5	3.3	2.3
Sole hemorrhage (SH)	4.8	4.3	3.4	5.3	6.9	8.1	9.8	10.5
Sole ulcer (SU)	7.0	4.1	5.7	8.6	11.8	15.1	15.9	17.0
Toe ulcer (TU)	1.6	1.0	1.6	2.1	2.3	2.6	2.4	3.2
White line lesion (WL)	5.4	3.1	4.4	6.9	9.3	10.9	12.7	16.6
Other lesions[1]	3.3	2.8	3.3	3.5	3.8	4.9	4.9	4.2
Any lesion	37.8	33.6	38.2	40.1	42.0	45.1	45.2	47.5

[1]Corkscrew claw, heel erosion, foot rot, axial fissure, vertical fissure, horizontal fissure, thin sole and unidentified lesions.

Table 2. Percentage of hoof trimming records with hoof lesions including only the first trimming session of the lactation from all the cows that were in the herds during the trimming period (Group 2), and stratified by parity

	All	Parity						
		1	2	3	4	5	6	≥ 7
Records (n)	104,446	39,786	28,594	18,033	9,936	4,950	2,125	1,022
Digital dermatitis (DD)	14.1	16.2	16.7	13.3	10.3	8.1	7.5	6.4
Interdigital dermatitis (ID)	1.3	1.3	1.6	1.5	1.4	1.4	0.7	0.9
Interdigital hyperplasia (IH)	1.4	0.9	1.7	2.0	2.0	1.7	2.4	1.7
Sole hemorrhage (SH)	3.4	3.2	2.5	3.8	5.0	5.7	7.0	7.6
Sole ulcer (SU)	4.9	3.0	4.2	6.1	8.6	10.6	11.4	12.4
Toe ulcer (TU)	1.1	0.7	1.2	1.5	1.7	1.8	1.7	2.3
White line lesion (WL)	3.8	2.3	3.2	5.0	6.8	7.6	9.1	12.1
Other lesions[1]	2.3	2.1	2.4	2.5	2.8	3.4	3.5	3.0
Any lesion	26.6	25.0	27.7	28.7	30.4	31.5	32.4	34.6

[1]Corkscrew claw, heel erosion, foot rot, axial fissure, vertical fissure, horizontal fissure, thin sole and unidentified lesions.

Table 3. Heritability on the observed scale (h^2) and heritability on the underlying scale (h_u^2) from univariate linear animal model when only hoof trimmed cows where considered (Group1), and when all cows present in the herd at time of hoof trimmer visit were included (Group2).

Trait	h^2		h_u^2	
	Group1	Group2	Group1	Group2
Digital dermatitis (DD)	**0.067** (0.007)	**0.053** (0.005)	**0.14** (0.01)	**0.13** (0.01)
Interdigital dermatitis (ID)	**0.015** (0.003)	**0.011** (0.002)	**0.13** (0.03)	**0.13** (0.02)
Interdigital hyperplasia (IH)	**0.036** (0.005)	**0.025** (0.004)	**0.29** (0.04)	**0.27** (0.04)
Sole hemorrhage (SH)	**0.017** (0.003)	**0.012** (0.002)	**0.08** (0.01)	**0.07** (0.01)
Sole ulcer (SU)	**0.038** (0.006)	**0.031** (0.004)	**0.14** (0.02)	**0.14** (0.02)
Toe ulcer (TU)	0.006 (0.002)	0.004 (0.001)	0.06 (0.02)	0.05 (0.03)
White line lesion (WL)	**0.017** (0.004)	**0.012** (0.002)	**0.07 (0.02)**	**0.06** (0.01)
Other lesion	0.007 (0.002)	0.005 (0.002)	0.04 (0.01)	0.04 (0.01)
Any lesion	**0.065** (0.007)	**0.048** (0.005)	**0.11** (0.01)	**0.09** (0.01)

[1]Corkscrew claw, heel erosion, foot rot, axial fissure, vertical fissure, horizontal fissure, thin sole and unidentified lesions.

Table 4. Genetic correlations from bivariate linear animal models only the first trimming session of the lactation from cows that has been visited at list one time by the trimmer during the lactation (Group 1),

Trait	ID	IH	SH	SU	TU	WL
Digital dermatitis (DD)	**0.5**4(0.11)	0.57(0.08)	0.04(0.11)	0.07(0.09)	-0.13(0.16)	**-0.30(0.10)**
Interdigital dermatitis (ID)		**0.61**(0.12)	0.12(0.15)	-0.13(0.14)	-0.29(0.21)	**-0.41(0.14)**
Interdigital hyperplasia (IH)			0.04(0.12)	0.20(0.10)	-0.21(0.19)`	-0.15(0.12)
Sole hemorrhage (SH)				**0.80(0.08)**	0.22(0.21)	**0.52(0.13)**
Sole ulcer (SU)					**0.56(0.14)**	**0.75(0.08)**
Toe ulcer (TU)						**0.58(0.16)**

[1] WL: White line lesion

Table 5. Genetic correlations from bivariate linear animal models when all cows present in the herd at time of hoof trimmer visit were included (Group 2).

Trait	ID	IH	SH	SU	TU	WL[1]
Digital dermatitis (DD)	**0.54(0.10)**	**0.60(0.08)**	0.08(0.11)	0.13(0.08)	-0.11(0.16)	**-0.23(0.10)**
Interdigital dermatitis (ID)		**0.61(0.11)**	0.11(0.14)	-0.07(0.13)	-0.24(0.21)	**-0.26(0.14)**
Interdigital hyperplasia (IH)			0.07(012)	-0.01(0.04)	-0.18(0.18)	-0.11(0.12)
Sole hemorrhage (SH)				**0.83(0.07)**	0.25(0.21)	**0.54(0.13)**
Sole ulcer (SU)					**0.60(0.14)**	**0.79(0.07)**
Toe ulcer (TU)						**0.54(0.17)**

[1] WL:White line lesion

Table 6. Heritability (h^2) from univariate linear animal model when all cows present in the herd at time of hoof trimmer visit were included (Group2), coding lesions as binary variables (0; 1), where 1 was assigned to the presence of a lesion (Binary), or using a severity score from 0 to 3 (Severity), and EBVs correlations of all sire (EBV), and of sire with at least 20 daughters (EBV20).

Trait	h^2		EBVs Correlation	
	Binary	Severity	EBV	EBV20
Digital dermatitis (DD)	**0.053** (0.005)	**0.052** (0.005)	0.94	0.96
Interdigital dermatitis (ID)	**0.011** (0.002)	**0.009** (0.002)	0.87	0.94
Interdigital hyperplasia (IH)	**0.025** (0.004)	**0.026** (0.004)	0.95	0.96
Sole hemorrhage (SH)	**0.012** (0.002)	**0.015** (0.003)	0.95	0.96
Sole ulcer (SU)	**0.031** (0.004)	**0.023** (0.004)	0.97	0.95
Toe ulcer (TU)	0.004 (0.001)	0.005 (0.002)	0.97	0.96
White line lesion (WL)	**0.012** (0.002)	**0.012** (0.003)	0.93	0.95

Figure 1. Manhattan plot showing markers and chromosomal regions associated with the Digital Dermatitis.

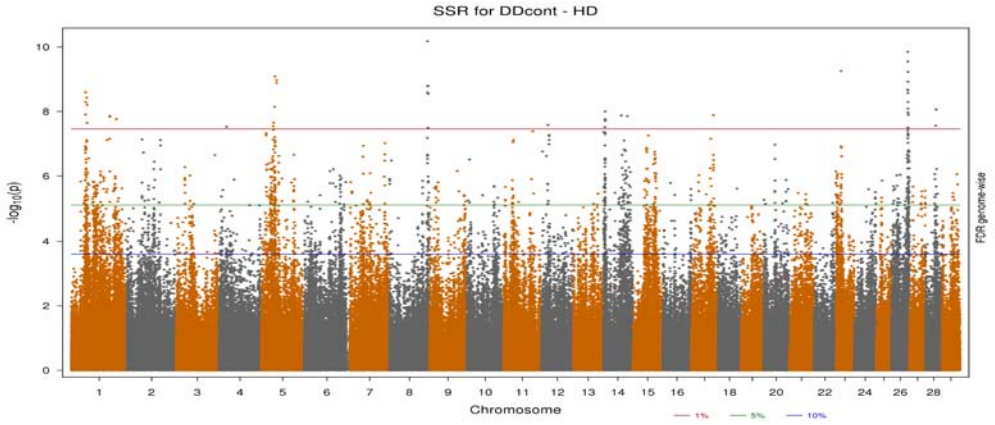


Figure 2. Manhattan plot showing markers and chromosomal regions associated with the Interdigital Hyperplasia.

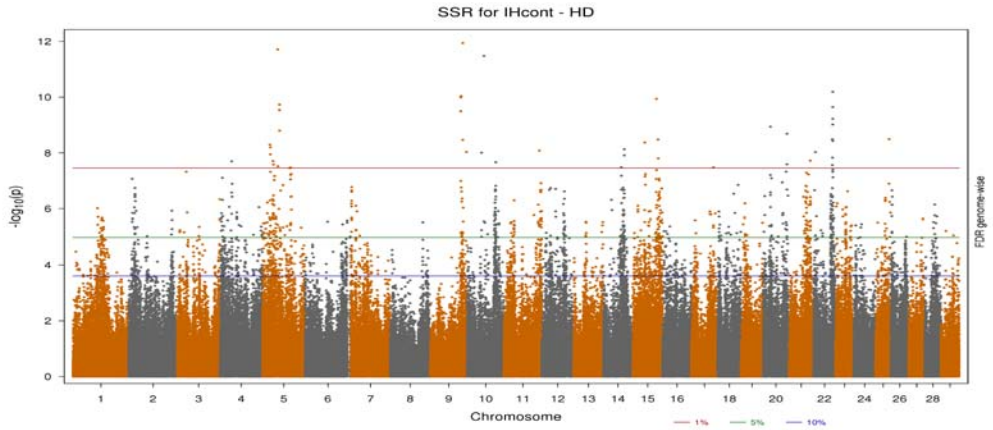
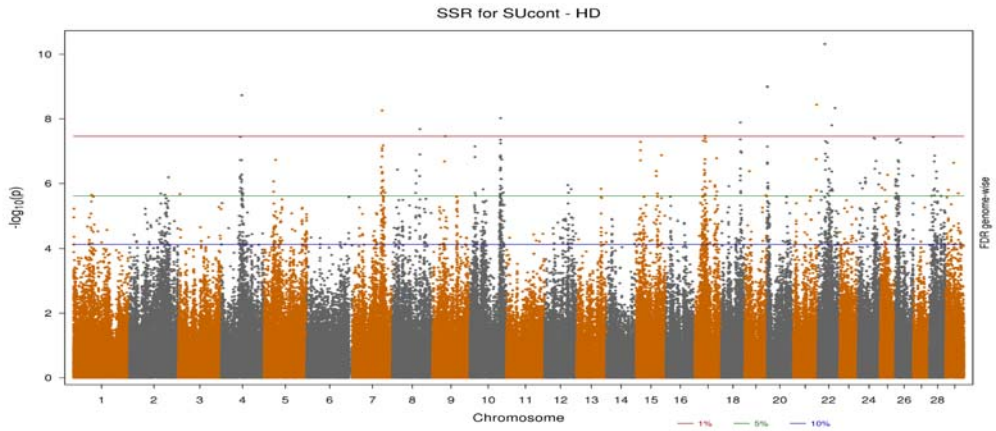


Figure 3. Manhattan plot showing markers and chromosomal regions associated with Sole Ulcer.



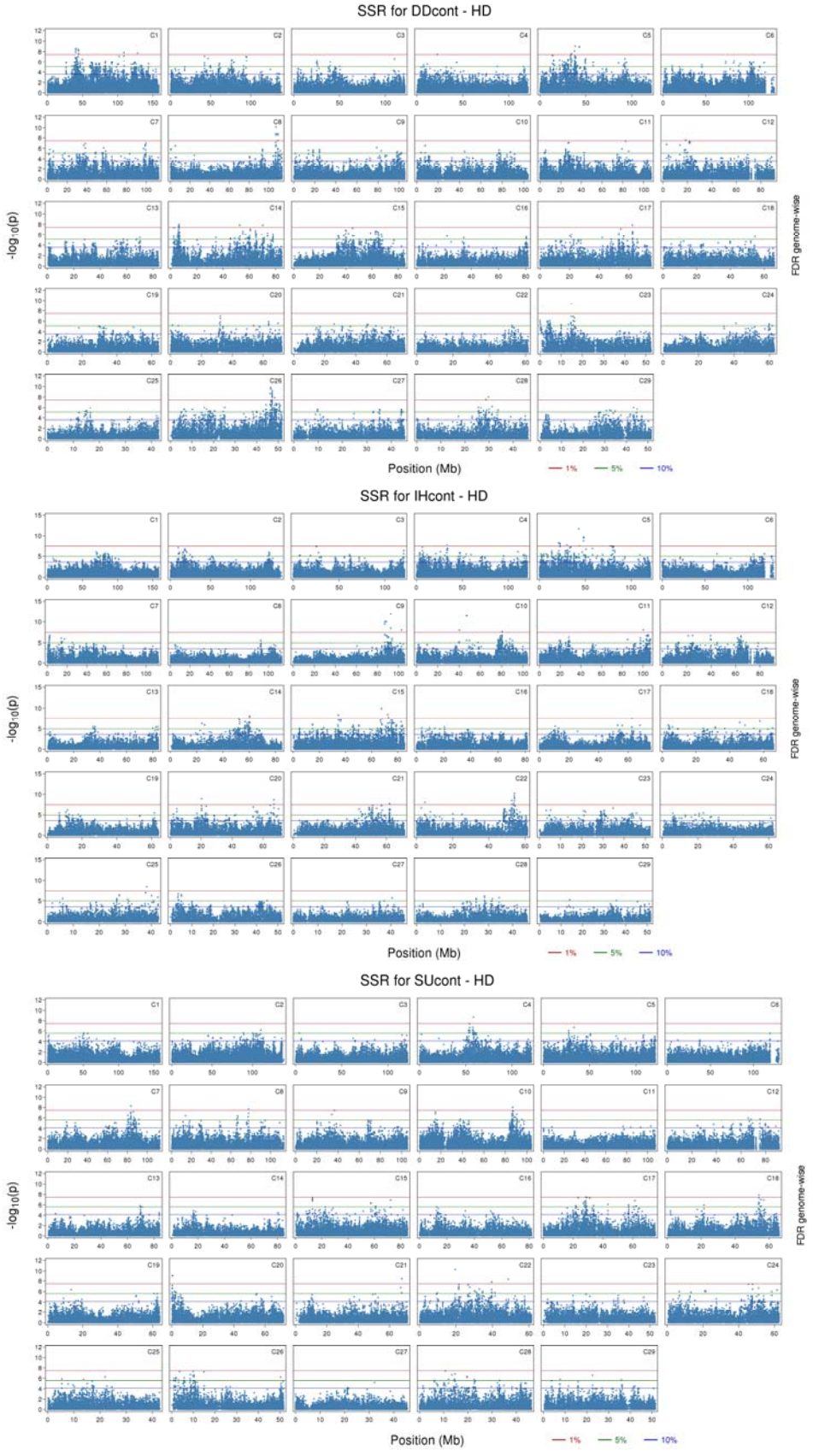

SSR for DDcont - HD
SSR for IHcont - HD
SSR for SUcont - HD
-log10(p)
Position (Mb)
FDR genome-wise
1%
5%
10%

Figure 4. Relationship between sire EBV for resistance to Digital Dermatitis and Sole Ulcer and percentage of healthy daughters for Group1 (n = 368 sires with at least 20 daughters) and 2 (n = 530 sires with at least 20 daughters)