Osteochondrosis in Duroc pigs scored by computed tomography: heritabilities based on genomic and pedigree relationship matrices

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ABSTRACT: The main objective of this study was to estimate the heritability of osteochondrosis by using phenotypes from an established method based on computed tomography (CT). Furthermore, validation of including genomic information was performed by comparing estimated heritability for the trait by using pedigree information (\(h^2_\lambda\)) and by using the combined pedigree and genomic relationship between the animals (\(h^2_G\)). Estimates were based on phenotypes (n=1882) and genotypes (n=2857) from Duroc boars. The scoring of OC was performed by assigning scores (0-5) on eight anatomical locations based on images from the CT, and the trait OCT was the sum score of these. Results show a minor increase in the heritability estimates by including the genomic relationship (\(h^2_\lambda=0.24, h^2_G=0.27\)). We conclude that CT is a suitable tool for assessing OC, and partial replacement of the pedigree-based relationship matrix by a genomic based relationship matrix will result in increased heritability.

Keywords: computed tomography; osteochondrosis; genomic relationship

Introduction

Osteochondrosis and computed tomography. Osteochondrosis (OC) is a developmental disorder known to cause lameness in pigs (Reiland, 1978). The disorder is a disturbance in the process of endochondral ossification caused by ischemia (Ytrehus et al., 2004, Olstad et al., 2011). Necrosis of the epiphyseal growth cartilage is the first structural change of OC, and can cause abnormalities of cartilage and thereafter bone lesions as seen in Figure 1. The condition has traditionally been assessed by either macroscopically scoring of the cartilage or by means of sectioning the bone and scoring the slabs from slaughter pigs. Computed tomography (CT) utilizes the ability of different body tissues to attenuate X-rays (Hounsfield, 1973), and images are constructed using a reconstruction of the attenuation profiles. The attenuation value is presented as a CT number (Hounsfield unit (HU)) in which soft/cartilaginous bone has a lower value (HU ~ 200) compared to calcified bone (HU~1000). Empel and Sehested (1986) utilized CT to evaluate osteochondral lesions of pigs post-mortem and demonstrated that osteochondrotic lesions can be seen as increased radiolucency in the affected areas. Recently, a method for scoring of osteochondrosis based on CT was proposed by Aasmundstad et al. (2013).

Genomic relationship. Traditionally, the relationship between animals has been calculated based on gene flow through pedigrees (Lynch and Walsh, 1997). In recent years, the single nucleotide polymorphisms (SNP) have been suggested used to calculate the relationship between animals (Miszta et al., 2013). Several methods for utilizing the genomic information in breeding value estimations have been proposed. One of the suggested methods is the single-step methodology, which combines a relationship matrix H constructed from a genomic relationship matrix G and a pedigree-based matrix A (Meuwissen et al., 2013).

Materials and Methods

Animals and phenotypes. Our material consists of Duroc boars tested on the central Norsvin boar test station in the period from January 2009 to January 2014. The purebred Duroc boars were born and raised to approximately 30 kg live weight in eight nucleus herds and thereafter sent to the boar test station. At the test station, the boars were penned in groups of 12, and fed ad libitum on conventional concentrate during the test period. The test ended at ~120 kg, and the boars were CT-scanned at this stage. Prior to the scanning, the boars were sedated using an intramuscular injection of Azaperone (Stresnil Vet®, Janssen Pharm). The scanning procedure took about five minutes for each animal and yielded output images for each 1.25

Figure 1. Photo from macroscopical inspection of the distal end of the humerus of a Duroc boar. A loose cartilage flap can be observed, originating from lesion “B” and CT-images of the same lesions. Letters indicate the same lesions. This boar was classified with a score of 3 on the medial condyle and a score of 4 on the lateral condyle. (Photo: P.O. Hofmo, Norsvin)
mm with a pixel size of 0.93 mm². For the 1,882 boars in the data material, the CT images were classified for signs of OC on a 5-point scale where the value 0 was assigned to animals with no sign of osteochondrosis and the value of 4 was assigned to animals where loose bone fragments were present (Aasmundstad et al., 2013). The classification was performed by utilizing the OsiriX software (Rosset et al., 2004), which facilitated a 2D-multiplar view. With this software the assessor could see the 2D images of the boar in the axial, sagittal and coronal planes simultaneously. A threshold window level of 400 HU and a window width of 400 HU were set to get the best contrast level. The assessor classified the boars for OC in eight anatomical locations: medial and lateral condyles at the distal end of the humerus and the femur of the right and left leg. OCT is the sum score of these eight scores for each boar, and the scoring procedure took less than five minutes per animal. The lowest possible score was 0 while the highest possible score was 40. The distribution of animals with phenotypes were skewed, hence an ln-transformation of data was done (Ln OCT). To facilitate this, the value 1 was added to all the phenotypes (OCT+1).

**Pedigree relationship.** The full pedigree information for all boars was provided by Norsvin. A criterion for the inclusion of an animal in the pedigree was that the birthdate should be from 1st of January 2002 and onwards, translating to at least five generations for boars with phenotypes.

**Genomic relationship.** Of the 1,881 boars with a phenotype for OC, 1,648 were genotyped. Additionally, 1,209 previously genotyped relatives were included in the construction of genomic relationship matrix (G). The genotyping of the 60K porcine SNP array was performed using the iScan platform (Illumina, San Diego, CA, USA). Genotypes were included in the genomic relationship analysis if the SNP markers passed quality thresholds of minor allele frequency, call frequency and Parent-Child Mendelian errors. A total of 38,453 of the SNPs were passing the quality control. Missing genotypes were imputed and genotype probabilities for ungenotyped animals calculated by using LDMIP (Meuwissen and Goddard, 2010). The genomic relationship matrix was constructed by using the second method presented in VanRaden (2008). In the end, the H⁻¹ relationship matrix combining the relationship matrices from the genotyped (G) and ungenotyped (A) animals were included in the genetic analysis software package DMU (Madsen and Jensen, 2008) (GBLUP).

**Model.** The statistical model fitted for OCT included the fixed effects of parity of the dam (1, 2 ≥ 3), herd and year of birth (34 levels), the five assessors while the random effects included in the model were pen and animal.

**Estimation of variance components, BLUE and BLUP.** Variance components were estimated using the DMU software (Madsen and Jensen, 2008) where the subroutine DMUAI was used for both the additive- and genonomic-based estimates. For inclusions of the genomic relationship the PG-MIX option in the software was utilized. The software also yielded output of BLUE and BLUP estimates.

**The validation of EBV vs. GEBV.** To test a potential benefit of using the GBLUP method, the phenotype from 200 random animals was masked. Breeding values based on traditional relationships (EBV), as well as on the combination of the genomic relationship and traditional relationship (GEBV) were calculated. For both EBV and GEBV the variance components were based on the additive relationship. Based on the estimates from the two analyses, a phenotype for each animal was predicted using the BLUE and BLUP values and a correlation was calculated between observed phenotype and the two phenotypes predicted from the BLUE and BLUP values.

**Results and Discussion**

The average OCT score of the 1,882 boars was 3.37, while the standard deviation of the observations was 2.63. The highest score obtained was 18, with the percentage of animals with a total score presented in Figure 2. A total of 10.5% of the boars were completely free of signs of lesions, while >90% had OCT < 8.

![Figure 2. Percentage of animals (n=1882) with total score of osteochondrosis](image-url)

The estimated heritability for the trait OCT based on pedigree was 0.24 (±0.21) as were also the case for the trait Ln OCT, while the estimated heritability for the same dataset using genomic relationship increased to 0.27. The estimates and corresponding standard errors are presented in table 1. The estimates based on the traditional pedigree is in good agreement with previously reported estimates published by Aasmundstad et al. (2013) who used CT, Jorgensen and Andersen (2000) who used X-ray and Lundeheim (1987) who examined sectioned bones. With the usage of CT as a tool for assessing OCT, a phenotype on a live animal is attained. With this selection program and this genetic variation a substantial increase in the pigs welfare will be achieved by reducing lameness.
Table 1. Estimates of genetic parameters for total osteochondrosis score for all eight locations estimated by use of traditional (AOCT) and genomic (GOCT) relationship

<table>
<thead>
<tr>
<th>Trait</th>
<th>$\sigma^2_A$</th>
<th>$\sigma^2_{PEN}$</th>
<th>$\sigma^2_E$</th>
<th>$h^2$</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOCT</td>
<td>1.46</td>
<td>0.26</td>
<td>4.37</td>
<td>0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>Ln_OCT</td>
<td>0.09</td>
<td>0.02</td>
<td>0.25</td>
<td>0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>GOCT</td>
<td>1.61</td>
<td>0.27</td>
<td>4.13</td>
<td>0.27</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Heritabilities calculated as var(a)/(var(a)+var(pen)+var(res))

Validation. As a test for the predictive ability of the EBV vs GEBV, a correlation between the EBV of OCT and the actual phenotype, as well as a correlation between the GEBV of OCT and the actual phenotype, were calculated. Figure 3 shows that the inclusion of the genomic relationship increased the correlation between the true and the predicted phenotype from 0.32 to 0.38 for the 200 animals, which would translate to an increased accuracy from 0.65 to 0.77. Notably, these estimates are influenced by the proportion of genotyped and non-genotyped animals and family structure. Nevertheless, the results are promising and the one-step genomic selection approach will improve selection for a reduction in the lameness related condition osteochondrosis.

Figure 3. Comparison of EBV and GEBV and the phenotype for 200 animals with masked phenotype

Conclusion

An in vivo assessment of osteochondrosis by using CT enables the phenotyping of live animals and the results show an increase in the heritability compared with traditional methods such as macroscopic examination or examination of sectioned bones. The inclusion of genomic relationship data will increase genetic gain in two ways: increasing the heritability of the trait and increasing the accuracy of selection.

Literature Cited