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# Genetic analyses of radiographic appearance of navicular bones in the Warmblood horse

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## Summary

Radiographic appearance of navicular bones (RNB) was analyzed genetically using the results of a standardized radiological examination of 5,157 Hanoverian Warmblood horses selected for sale at auction. Different categorisation schemes were used to describe RNB. Definition of RNB traits was based on type and/or extent of radiographic findings in the navicular bones of the front limbs. Analyses included pseudo-linear ($RNB_{0\text{-}3}$, $RNB_{0\text{-}7}$) and binary traits ($RNB_{0/1a\text{-}e}$). The most specific pseudo-linear trait ($RNB_{0\text{-}7}$) accounting for different types of radiographic findings served as reference trait for RNB. Deviation from normal RNB was classified for defining more general RNB traits. Genetic parameters were estimated multivariately with REML. Breeding values were predicted uni- and multivariately using PEST. Heritability estimates for navicular bone appearance were in the range of $h^2$ = 0.10 to $h^2$ = 0.33. Additive genetic correlations between RNB traits were positive and mostly larger than $r_g$ = 0.80. Binary coding based on existence or non-existence of marked radiological alterations in the navicular bones ($RNB_{0/1d}$) resulted in an additive genetic correlation of $r_g$ = 0.98 to the reference trait ($RNB_{0\text{-}7}$). Correlation between respective breeding values was r = 0.84. Higher heritability of the binary trait ($RNB_{0/1d}$; $h^2$ = 0,24) suggests that classification of RNB and use of binary coding could be beneficial when considering RNB in future breeding plans for the Warmblood horse.

## Introduction

Navicular bone is an important and unique part of equine skeleton. It serves as a deflexion plate for deep digital flexor tendon and is therefore involved in the regulation of load and traction forces in the distal part of the equine limb. Together with synovial (bursa podotrochlearis, distal interphalangeal joint), tendinous (distal part of deep digital flexor tendon) and ligamentous structures it forms the equine podotrochlea.

Considerable strain is put on the limbs of a horse, particularly on the front limbs. Correspondingly, lameness problems play an important role in horse medicine. Recurrent or persistent forehand lameness can often be located in the most distal part of the equine limb. Special radiographic projections might show alterations in the navicular bones of the front limbs considered indicative of the so-called podotrochlosis syndrome (navicular disease). However, the prognostic value of radiography has been questioned as there is considerable disagreement on interpretation of radiographic appearance of the navicular bones. Number, location and shape of canales sesamoidales, contour and structure of the navicular bone serve as diagnostic criteria when evaluating navicular bones radiologically, but varying importance is ascribed to the individual radiographic findings (DIK and VAN DEN BROEK 1995, HERTSCH and ZELLER 1976, UELTSCHI 2002). Several studies could not show any significant difference between distributions of radiographic findings in the navicular bones in clinically healthy and lame horses (AMMANN 1987, BODENMÜLLER 1983, BRANSCHEID 1977, LANGFELDT 1986, LEUENBERGER 1987, RÖSTEL-PETERS 1987, SEYREK-INTAS 1993). Presence of marked radiological alterations in the navicular bones involves a higher risk of clinically manifestation, but in many cases eventual clinical relevance, i.e. association between particular radiographic findings and the development of clinical signs of navicular disease, remains speculative (BRUNKEN 1986, SEYREK-INTAS 1993). Nevertheless, deviation from presumably normal radiographic appearance of the navicular bones is likely to take effect on the sales value of the individual horse (VAN HOOGMOED et al. 2003).

Genetic determination of navicular disease and of certain radiological characteristics of the navicular bones has been reported (ASTNER 1996, BOS et al. 1986, DIK and VAN DEN BROEK 1995, KWPN 1994, STOCK et al. 2004, WILLMS et al. 1999, WINTER et al. 1996). Based on investigations on the genetic background of and the genetic correlations between individual radiographic findings in the navicular bones form the basis of breeding strategies can be developed that account for the radiographic appearance of the navicular bones and will therefore counteract the adverse effects of navicular bone pathology.

## Material and Methods

The study was based on the results of a standardized veterinary medical examination of 5,157 Hanoverian Warmblood horses, selected for sale at riding horse auctions in 1997 to

2004 by the Society of Hanoverian Warmblood breeders (Verband hannoverscher Warmblutzüchter e.V., VHW) in Verden on the Aller, Germany. The horses had an age of between 3 and 11 years (mean age of 4.14 ± 0.86 years). Male to female ratio was about 3:2, with 141 stallions, 2,847 geldings and 2,169 mares selected for auction sale.

The radiological examination of the horses included lateromedial (90°) projections of all four feet and dorsoproximo-palmarodistal projections (upright pedal route according to OXSPRING 1935) of navicular bone region of the front feet. All radiographs were scrutinized by two experienced radiologists. Deviations from normal radiological appearance of bony structures were put down in individual veterinary records of the investigated horses. Diagnostic criteria referred to number, localization and shape of canales sesamoidales, and to structure and contour of the navicular bones of the front feet. Different categorization schemes were used to describe radiographic appearance of the navicular bones (RNB). Categories were defined as follows: 0 − no abnormal radiographic findings in the navicular bones of the front feet; I − few conical canales sesamoidales in the central part of the distal border; II − several conical canales sesamoidales in the central part of the distal border; III − single elongated and/or deformed (enlarged) canales sesamoidales in the central part of the distal border; IV − several elongated and/or deformed (enlarged) canales sesamoidales; V − diverse markedly deformed (enlarged, elongated, branched) canales sesamoidales in the central and/or medial or lateral part of the distal border; VI − alterations of navicular bone contour, i.e. new bone formation at proximal or distal border or at medial or lateral extremity of navicular bone; VII − alterations of navicular bone structure, i.e. irregular pattern of spongiosa and increased (sclerosis) or decreased (osteolysis) navicular bone density.

Two quasi-linear and five binary RNB traits were defined on the basis of type and/or extent of radiographic findings. In each case horses were classified according to the most distinct radiographic finding in left or right front navicular bone. The most specific RNB trait, i.e. the quasi-linear trait with seven categories, served as reference trait ($RNB_{0-7}$). For the definition of the second quasi-linear trait categories 3 to 7 were combined, so that it comprised only four categories (0, I, II, III-VII; $RNB_{0-3}$). All-or-none traits were defined in that way that horses were considered affected if they were allocated to category I ($RNB_{0/1a}$), to category II ($RNB_{0/1b}$), to categories III to VII ($RNB_{0/1c}$), or to categories II to VII ($RNB_{0/1d}$), or if they had any kind of radiological alterations at the distal border of the navicular bones ($RNB_{0/1e}$). In each case all other horses were considered as not affected in respect of this trait.

Pedigree data were taken from a unified animal ownership database (Vereinigte Informationssysteme Tierhaltung w.V., VIT) in Verden on the Aller, Germany. The 5,157 horses descended from 508 different sires that had on the average 10.15 ± 19.23 (range 1 to 251) investigated offspring. For the genetic analyses four generation pedigrees of all horses were considered, resulting in a relationship matrix comprising 23,279 horses.

Genetic parameters were estimated multivariately in linear animal models using Residual Maximum Likelihood (REML) with VCE-5, Version 5.1.2 (Variance Component Estimation; Kovac et al., 2003). The models of all RNB traits included the same fixed effects that were significant in the multivariate analyses of variance using the procedures GENMOD (binary traits) and GLM (quasi-linear linear traits) of the Statistical Analysis System (SAS), version 9.1.3 (SAS Institute, Cary, NC, 2005).

$$y_{ijklm} = \mu + Year_i + Sex_j * Age_k + a_l + e_{ijklm}$$

with $y_{ijklm}$ = radiographic finding of the horse, $\mu$ = model constant, $Year_i$ = fixed effect of the i-th year of auction selection (i = 1-8), $Sex_j * Age_k$ = fixed effect of the interaction between the j-th sex (j = 1-3) and the k-th age group (k = 1-4), $a_l$ = random additive genetic effect of the l-th animal, and $e_{ijklm}$ = residual.

Additive genetic ($\sigma^2_a$, $cov_a$) and residual ($\sigma^2_e$, $cov_e$) variance and covariance estimates were used to calculate heritabilities ($h^2$), additive genetic ($r_g$) and residual ($r_e$) correlations. Heritabilities and residual correlations of the binary traits were transformed onto the underlying liability scale according to Dempster and Lerner (1950) and Vinson et al. (1976). The (co)variance matrices and the same multivariate linear animal models as for the estimation of genetic parameters were further utilized to predict breeding values for the different RNB traits for all 23,279 horses in the last four generations of the pedigrees of the investigated horses using PEST (Groeneveld 1990). Using the 489 sires of investigated horses which were born after 1972 as the reference population, breeding values were standardized to a mean of 100 and a standard deviation of 20. Transformation of relative breeding values (RBV) was performed that way that horses with higher RBV are considered to transmit a lower disposition, horses with lower RBV are considered to transmit a higher disposition for particular radiographic findings in the navicular bones.

Correlations between RBV for the different RNB traits were investigated using Pearson correlation coefficients of the procedure CORR of the Statistical Analysis System (SAS), version 9.1.3 (SAS Institute, Cary, NC, 2005).

## Results

Distributions of trait levels of the two quasi-linear and the five binary RNB traits are given in Table 1. Deviation from normal RNB occurred in 40.57% of the horses, mostly in form of few (20.48%) or several (16.97%) conical canales sesamoidales in the central part of the distal border of the navicular bones of the front feet. Radiographic findings were almost exclusively (in 98.6% of the horses with radiographic findings) located in the distal border of the navicular bones.

TABLE 1: Prevalences of quasi-linear and binary traits referring to the radiographic appearance of the navicular bones of the front feet (RNB) in 5,157 Warmblood riding horses selected for sale at auction

| Type of trait | Trait designation | Coding | RNB category | Prevalence absolute | Prevalence (relative) |
|---|---|---|---|---|---|
| Quasi-linear | $RNB_{0-7}$ | 0 | 0 | 3065 | (59.43%) |
| | | 1 | I | 1056 | (20.48%) |
| | | 2 | II | 875 | (16.97%) |
| | | 3 | III | 65 | (1.26%) |
| | | 4 | IV | 17 | (0.33%) |
| | | 5 | V | 33 | (0.64%) |
| | | 6 | VI | 26 | (0.50%) |
| | | 7 | VII | 20 | (0.39%) |
| | $RNB_{0-3}$ | 0 | 0 | 3065 | (59.43%) |
| | | 1 | I | 1056 | (20.48%) |
| | | 2 | II | 875 | (16.97%) |
| | | 3 | III-VII | 161 | (3.12%) |
| Binary | $RNB_{0/1a}$ | 0 | 0 + II-VII | 4101 | (79.52%) |
| | | 1 | I | 1056 | (20.48%) |
| | $RNB_{0/1b}$ | 0 | 0 + I + III-VII | 4282 | (83.03%) |
| | | 1 | II | 875 | (16.97%) |
| | $RNB_{0/1c}$ | 0 | 0 + I + II | 4996 | (96.88%) |
| | | 1 | III-VII | 161 | (3.12%) |
| | $RNB_{0/1d}$ | 0 | 0 + I | 4121 | (79.91%) |
| | | 1 | II-VII | 1036 | (20.09%) |
| | $RNB_{0/1e}$ | 0 | 0 | 3093 | (59.98%) |
| | | 1 | I-VII (distal border) | 2064 | (40.02%) |

RNB categories: 0 − no abnormal radiographic findings in the navicular bones of the front feet; I − few conical canales sesamoidales in the central part of the distal border; II − several conical canales sesamoidales in the central part of the distal border; III − single deformed (enlarged and/or elongated) canales sesamoidales in the central part of the distal border; IV − several deformed (enlarged and/or elongated) canales sesamoidales; V − diverse markedly deformed (enlarged, elongated, branched) canales sesamoidales in the central and/or medial or lateral part of the distal border; VI − alterations of navicular bone contour, i.e. new bone formation at proximal or distal border or at medial or lateral extremity of navicular bone; VII − alterations of navicular bone structure, i.e. irregular pattern of spongiosa and increased (sclerosis) or decreased (osteolysis) navicular bone density.

Genetic parameters estimated for the different RNB traits are given in Table 2. Heritability estimates for RNB were in the range of $h^2 = 0.10$ to $h^2 = 0.33$, with standard errors of $SE_{h^2} = 0.02\text{-}0.09$. Additive genetic correlations between the RNB traits were positive and mostly larger than $r_g = 0.80$ ($SE_{rg} = 0.01\text{-}0.25$). The additive genetic correlation to the reference trait

($RNB_{0-7}$) was lowest for $RNB_{0/1c}$ ($r_g$ = 0.60) and highest for $RNB_{0/1d}$ ($r_g$ = 0.98). Residual correlations ranged between $r_e$ = -0.37 and $r_e$ = 0.94 ($SE_{re} \leq 0.01$) before transformation. Transformation resulted in estimates of residual correlations which were partly out off parameter space.

TABLE 2: Heritabilities (transformed estimates for the binary traits; bold on the diagonal), additive genetic correlations (above the diagonal) and residual correlations (transformed estimates for the binary traits; below the diagonal) with their standard errors for the two quasi-linear ($RNB_{0-7}$, $RNB_{0-3}$) and the five binary traits ($RNB_{0/1a-e}$) referring to the radiographic appearance of the navicular bones of the front feet (RNB) in 5,157 Warmblood riding horses selected for sale at auction

| | $RNB_{0-7}$ | $RNB_{0-3}$ | $RNB_{0/1a}$ | $RNB_{0/1b}$ | $RNB_{0/1c}$ | $RNB_{0/1d}$ | $RNB_{0/1e}$ |
|---|---|---|---|---|---|---|---|
| $RNB_{0-7}$ | **0.138 ± 0.021** | 0.983 ± 0.007 | 0.848 ± 0.090 | 0.924 ± 0.046 | 0.596 ± 0.129 | 0.977 ± 0.016 | 0.943 ± 0.024 |
| $RNB_{0-3}$ | 0.940 ± 0.002 | **0.180 ± 0.023** | 0.881 ± 0.073 | 0.993 ± 0.024 | 0.407 ± 0.152 | 0.995 ± 0.008 | 0.984 ± 0.008 |
| $RNB_{0/1a}$ | 0.106 ± 0.020 | 0.184 ± 0.020 | **0.099 ± 0.025** | 0.876 ± 0.133 | 0.074 ± 0.248 | 0.826 ± 0.114 | 0.939 ± 0.047 |
| $RNB_{0/1b}$ | 0.751 ± 0.016 | 0.973 ± 0.013 | -0.731 ± 0.030 | **0.248 ± 0.036** | 0.235 ± 0.219 | 0.980 ± 0.014 | 0.992 ± 0.024 |
| $RNB_{0/1c}$ | 1.685 ± 0.021 | 1.309 ± 0.027 | -0.332 ± 0.041 | -0.334 ± 0.047 | **0.151 ± 0.092** | 0.422 ± 0.187 | 0.262 ± 0.160 |
| $RNB_{0/1d}$ | 1.116 ± 0.008 | 1.218 ± 0.006 | -0.745 ± 0.027 | 1.887 ± 0.006 | 1.316 ± 0.041 | **0.238 ± 0.036** | 0.968 ± 0.023 |
| $RNB_{0/1e}$ | 0.861 ± 0.011 | 1.045 ± 0.007 | 1.082 ± 0.016 | 0.866 ± 0.023 | 0.532 ± 0.044 | 0.917 ± 0.023 | **0.330 ± 0.038** |

For trait definitions see TABLE 1.

In the 508 sires of investigated horses breeding values for the quasi-linear RNB traits ranged between -0.85 and 2.36 ($RNB_{0-7}$) and -0.63 and 1.42 ($RNB_{0-3}$) and for the binary RNB traits $RNB_{0-7}$ ($RNB_{0/1a-e}$) between -0.40 and 0.78. Means, standard deviations and ranges of RBV for the RNB traits in the sires and in all horses appearing in the last four generations of investigated horses are shown in Table 3. Mean RBV were in all cases lower in whole four generation pedigree than in the sires. RBV ranges were in most cases smaller in the sires than in the whole pedigree. However, minimum and maximum RBV for two of the binary RNB traits were identical ($RNB_{0/1b}$) or very similar ($RNB_{0/1d}$) in the sires and in the whole pedigree.

TABLE 3: Distribution of relative breeding values (RBV) for the quasi-linear and binary traits referring to the radiographic appearance of the navicular bones of the front feet (RNB) among all 23,279 horses appearing in the last four generations of investigated horses and among the 508 sires of investigated horses

| Trait | Four-generation-pedigree (n = 23,279) | | | Sires of investigated horses (n = 508) | | |
|---|---|---|---|---|---|---|
| | mean ± SD | minimum | maximum | mean ± SD | minimum | maximum |
| $RNB_{0-7}$ | 97.50 ± 19.63 | -115.56 | 159.88 | 100.15 ± 20.12 | -52.37 | 152.40 |
| $RNB_{0-3}$ | 97.49 ± 19.62 | -12.36 | 163.33 | 100.11 ± 20.26 | -8.87 | 147.40 |
| $RNB_{0/1a}$ | 98.73 ± 20.71 | -13.34 | 169.05 | 100.15 ± 20.15 | 13.78 | 157.92 |
| $RNB_{0/1b}$ | 98.25 ± 20.38 | -51.20 | 153.55 | 100.04 ± 20.26 | -51.20 | 153.55 |
| $RNB_{0/1c}$ | 98.94 ± 20.46 | -104.10 | 150.04 | 100.03 ± 19.94 | -92.43 | 139.27 |
| $RNB_{0/1d}$ | 97.93 ± 19.96 | -33.91 | 155.34 | 100.05 ± 20.12 | -33.91 | 152.72 |
| $RNB_{0/1e}$ | 97.67 ± 19.85 | 19.93 | 171.67 | 100.12 ± 20.38 | 32.45 | 152.94 |

SD − standard deviation. For trait definitions see TABLE 1.

Correlations between most of the RBV were significantly positive with Pearson correlation coefficients ranging between r = 0.23 and r = 0.96 (Table 4). $RBV_{RNB0/1d}$ and $RBV_{RNB0/1e}$ were closely correlated with r = 0.80-0.91 to the RBV predicted for the two quasi-linear RNB traits ($RBV_{RNB0-7}$, $RBV_{RNB0-3}$).

TABLE 4: Pearson correlation coefficients (significant correlations coefficients in bold) between the relative breeding values (RBV) predicted for the quasi-linear and binary trait referring to the radiographic appearance of the navicular bones of the front feet (RNB)

| Trait | $RBV_{RNB0-7}$ | $RBV_{RNB0-3}$ | $RBV_{RNB0/1a}$ | $RBV_{RNB0/1b}$ | $RBV_{RNB0/1c}$ | $RBV_{RNB0/1d}$ | $RBV_{RNB0/1e}$ |
|---|---|---|---|---|---|---|---|
| $RBV_{RNB0-7}$ | 1.000 | **0.958** $^{***}$ | **0.275** $^{***}$ | **0.598** $^{***}$ | **0.679** $^{***}$ | **0.843** $^{***}$ | **0.803** $^{***}$ |
| $RBV_{RNB0-3}$ | | 1.000 | **0.352** $^{***}$ | **0.738** $^{***}$ | **0.497** $^{***}$ | **0.894** $^{***}$ | **0.908** $^{***}$ |
| $RBV_{RNB0/1a}$ | | | 1.000 | -0.057 | -0.038 | -0.070 | **0.675** $^{***}$ |
| $RBV_{RNB0/1b}$ | | | | 1.000 | -0.045 | **0.904** $^{***}$ | **0.633** $^{***}$ |
| $RBV_{RNB0/1c}$ | | | | | 1.000 | **0.387** $^{***}$ | **0.232** $^{***}$ |
| $RBV_{RNB0/1d}$ | | | | | | 1.000 | **0.684** $^{***}$ |
| $RBV_{RNB0/1e}$ | | | | | | | 1.000 |

Levels of significance: $^{***}$ – $P < 0.001$; $^{**}$ – $P < 0.01$; $^{*}$ – $P < 0.05$; $^{+}$ – $P < 0.10$.
For trait definitions see TABLE 1.

## Conclusions

Genetic determination of the radiographic appearance of the navicular bones of the front feet of Warmblood riding horses has been substantiated. Use of binary coding, based on existence or non-existence of marked radiological alterations in the navicular bones or of any kind of radiological alterations in the distal border of the navicular bones, resulted in heritability estimates exceeding those for more specifically defined quasi-linear traits. At the same time, respective additive genetic correlations were highly positive as were correlations between corresponding breeding values. Consideration of the radiographic appearance of the navicular bones of the front feet, using either quasi-linear or appropriate binary trait definition, in future breeding plans of the Warmblood horse will allow for effective selection for absence of marked radiological alterations in the navicular bones of young horses.

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