Inbreeding and Melanoma in Lipizzan Horses

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SUMMARY

The relationship between inbreeding and melanoma status (graded from 0 to 4) was analysed by various regression models. Analysed data referred to 296 grey Lipizzan horses originating from five state-owned studs (Austria, Croatia, Hungary, Slovakia and Slovenia) and with average inbreeding coefficient (F=0.107) calculated from extremely informative pedigrees (98% and 76% of horses had completely full pedigree in generation 10 and 20, respectively). In all regression models, in addition, the effects of stud (fixed) and age (covariate) were included. When all data were treated as one population, the estimates from linear and ancestral inbreeding models were not significant. Total inbreeding effect estimates (at F=0.125 and Fa=0.57) were 0.26 and 0.30 for the ancestral inbreeding and linear regression models, respectively. Heterogeneity among state-owned studs in inbreeding effects was also tested for both models and weak statistical significance was obtained for the interaction model with ancestral inbreeding (P=0.049). However, observed effect in the model with interaction was not consistent, did not yield in better model fitting and the obtained significance is probably just a statistical artefact. In general, although some indications about the relationship between ancestral inbreeding and melanoma were present, inbreeding does not appear to be a factor that substantially influences the expression of melanoma in Lipizzan horses.

KEY WORDS

Ancestral inbreeding, Inbreeding, Lipizzan horse, Melanoma

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Povezanost inbreedinga i melanoma kod lipicanskih konja

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SAŽETAK

Regresijskim modelima analizirana je povezanost stadija melanoma (na skali od 0 do 4) i inbreedinga. Analizirani podaci odnose se na 296 sivih lipicanskih konja iz pet državnih ergela (Austrija, Hrvatska, Mađarska, Slovačka i Slovenija). Prosječna razina inbreedinga (F=0.107) izračunata je na temelju izuzetno informativnog pedigreea (98% jedinki imalo je potpuno poznat pedigre u generaciji 10, a 76% jedinki imalo je potpuno poznat pedigree u generaciji 20). U svim modelima uvažen je i utjecaj ergele (fiksni utjecaj) te starosti konja (kovarijabla). Kada se analiza koeficijenta regresije (regresija stadija melanoma na koeficijent inbreedinga) odnosila na populaciju kao jednu cjelinu, procjena linearnog koeficijenta regresije za inbreeding kao i za inbreeding predaka nije bila signifikantna. Kod razine inbreedinga F=0.125 i Fa=0.57, ukupna procjena linearnog utjecaja inbreedinga bila je 0.30, a utjecaja inbreedinga predaka bila je 0.26. Analizirana je i heterogenost utjecaja inbreedinga između državnih ergela i za interakcijski model sa inbreedingom predaka dobivena je slaba signifikantna interakcija (P=0.049). Ipak, dobiveni utjecaj u model sa interakcijom nije bio konzistentan, nije bolje objašnjavao nerazjašnjenu varijabilnost modela te je dobivena signifikantnost vjerojatno posljedica slučajnosti. Iz navedenog se može zaključiti, premda postoji indicija o povezanosti inbreedinga predaka i melanoma, da kod lipicanskih konja inbreeding nije čimbenik koji utječe na pojavu melanoma.

KLJUČNE RIJEČI

inbreeding predaka, inbreeding, lipicanac, melanom

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INTRODUCTION

The Lipizzan horse breed was established in 1580 in Lipica by the Imperial court in Vienna with intention of providing nobility and royal prestige in the equestrian arts. This predominantly grey and "baroque" horse was originally kept all over what was once Austro-Hungarian Empire and today is a part of the European cultural heritage. Currently, the breed numbers between 2000 and 3000 horses that are bred in the state-owned studs in Austria, Bosnia and Herzegovina, Croatia, Hungary, Italy, Romania, Slovakia, Slovenia and Yugoslavia as well as by private owners all over the world. The studbooks were mainly closed during the breeding history and today, due to remote connections in the pedigrees, mating of related individuals is unavoidable.

Equine melanoma is a form of skin cancer that is characteristically showing high incidence in grey horses with ageing. For the Lipizzan horse, melanoma is a frequent disease with notable detrimental influence on the health and aesthetic appearance of the horses. Although the genetic mode of the melanoma inheritance is still inconclusive, a genetic impact on the disease is strongly suggested (Rieder et al. 2000).

The objective of this study was to analyze the effects of inbreeding on melanoma. The effects of ancestral inbreeding on melanoma were also analyzed.

MATERIALS AND METHODS

Data included 296 grey breeding Lipizzan horses (older than three years) from the following five national studs: Đakovo (Croatia), Lipica (Slovenia), Piber (Austria), Szilvásvárd (Hungary) and Topol'čianky (Slovakia). All horses were examined and classified for melanoma status as described in Seltenhammer (2000), see Table 1. The same person was grading all horses. As gradation five was not observed the scale from zero to four was used.

Individual inbreeding coefficients (F) for all 296 horses as well as for their parents were calculated by the tabular method using the algorithm of VanRaden (1992). This algorithm enabled us to calculate individual inbreeding coefficients with restricted pedigree depth. For each horse, we also calculated the ancestral inbreeding coefficient, F_a.

According to Ballou (1997), ancestral inbreeding coefficient is defined as the cumulative proportion of an individual's genome that has been previously exposed to inbreeding in its ancestors:

$$F_a = [F_{a(s)} + (1 - F_{a(s)})\, F_{(s)} + F_{a(d)} + (1 - F_{a(d)})\, F_{(d)}]\, /2, \quad (1)$$

where the subscripts s and d represent values for the sire and dam of that individual, respectively. Thus, for example, $F_{(s)}$ is inbreeding coefficient and $F_{a(s)}$ is ancestral inbreeding coefficient of the father of an individual. The effect of stud (fixed) and age (covariate) were included in the basic model. The interaction between stud and age as well as the effect of sex were omitted from the basic model, as they were not significant. Considering the basic model, several hypotheses, related to the relationship between inbreeding and melanoma, were tested by the following models;

Model I:

$$y = \mu + Stud_i + b\ Age + bf\ F + e, \quad (2)$$

Model II:

$$y = \mu + Stud_i + b\ Age + bf\ F + bfa\ F\ F_a + e, \quad (3)$$

Model III:

$$y = \mu + Stud_i + b\ Age + bf_i(Stud_i\ F) + e, \quad (4)$$

Model IV:

$$y = \mu + Stud_i + b\ Age + bf_i\ (Stud_i\ F) + + bfa_i\ (Stud_i\ F\ F_a) + e, \quad (5)$$

where y is the melanoma status of the individual horse (0, ..., 4); μ is the overall mean; $Stud_i$ is fixed effect of state stud i (i = 1, ... ,5); Age is the effect of age of the individual horse (here obtained as a difference between examination and birth year and was treated in models as a covariable); F is the inbreeding coefficient of the individual horse; F_a is the ancestral inbreeding coefficient of the individual horse; ($Stud_i$ F) is the interaction between state stud i and the individual inbreeding

Table 1. Clinical classification and incidence of melanoma in Lipizzan horses

Gradation	Description	Horses	%
Grade 0	Free of melanoma.	148	50.0
Grade 1	Early stages of plaque-type or one solitary nodule of maximal lens-size situated on typical locales.	22	7.4
Grade 2	Several nodules of maximal lens-size or one solitary nodus with size of a bean on typical locales.	61	20.6
Grade 3	One or several nodular melanomas of maximal size of an egg intra- and/or subcutan on typical locales (or lips)	49	16.6
Grade 4	Extensive confluent melanoma-nodules, covered with skin, signs of destruction and metastasis.	16	5.4
Grade 5	Exophytical growth, tumor nodules show wet surface, destruction, extensive metastasis into different organs accompanying by severe metabolic disorders.	0	0.0

coefficient; ($Stud_k$ F F_a) is the interaction between state stud k, individual inbreeding coefficient and ancestral individual inbreeding coefficient; bf and bfa are pooled regression coefficients (common slopes) and bf_i and bfa_i are individual regression coefficients by stud; e is the random residual effect of the individual horse.

Linear inbreeding depression was estimated with Model I. The Model II includes the ancestral inbreeding coefficient and as proposed by Ballou, (1997) it tests if inbreeding depression is modified by the level of ancestral inbreeding. Under the assumption that inbreeding depression has been purging, that is if negative inbreeding effects have been reduced, interaction effects of the inbreeding and ancestral inbreeding on the observed traits will be positive (negative in the present paper as higher melanoma grades are more detrimental for the health of an horse). The Model III tested heterogeneity of linear inbreeding depression among different studs. A similar approach was used in Miglior et al. (1994). We also tested the hypothesis that there is no difference between studs in the interaction effects of the ancestral inbreeding and inbreeding depression (see Model IV). Theoretical explanations for the existence of heterogeneity of inbreeding depression (effects) are given by Curik et al. (2001) for models with a finite number of loci and/or epistasis, as well as by Lerner (1954) for models assuming interaction between genotype and environment (theory of homeostasis). All statistical analyses were performed by PROC GLM (SAS 1989).

RESULTS AND DISCUSSION

Descriptive statistics for melanoma gradation, inbreeding level (F) and ancestral inbreeding level (Fa) among state studs are shown in Table 2. The overall inbreeding level (F = 0.107) was higher then the level observed in other horse populations (MacCluer et al. 1983; Klemetsdal 1993; Gandini et al. 1992; Moureaux et al. 1996). However, we should be aware that pedigree used in this study was extremely informative (see Figure 1) and, if compared with the same pedigree information, similar inbreeding level as in other studies would be obtained (see Figure 2).

When all data were treated as one population, estimated total inbreeding effects (TIE) with Model I as well as with Model II were positive (see Table 3). The estimates from the linear regression model were not significant. The estimates from ancestral inbreeding model were also not significant thus showing that a purging effect, as measured by ancestral inbreeding coefficient, was not present.

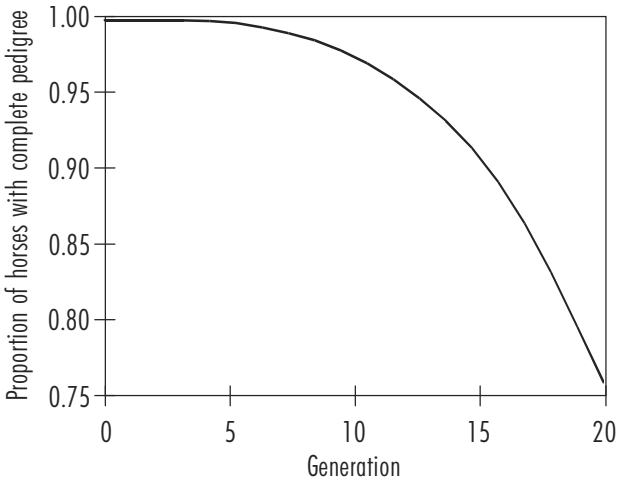

Figure 1. Proportion of horses with complete pedigree by generation

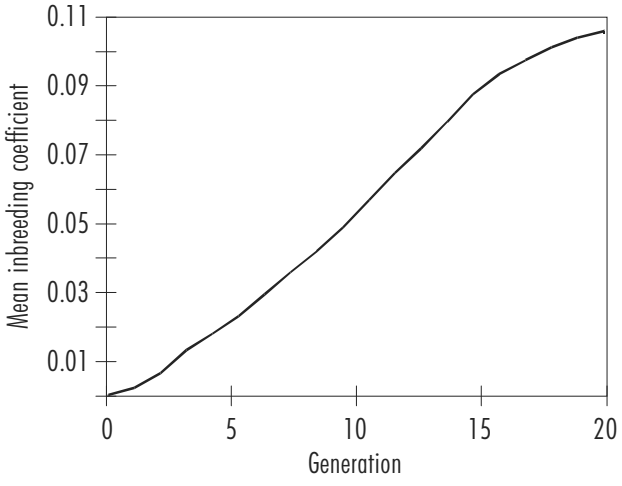

Figure 2. Mean inbreeding coefficient by generation

Table 2. Descriptive statistics for melanoma gradation, inbreeding level (F) and ancestral inbreeding level (Fa)

State stud	N	Melanoma gradation		F		Fa	
		Mean	SE	Mean	STD	Mean	STD
Đakovo	33	1.877	0.205	0.091	0.031	0.554	0.020
Lipica	52	1.649	0.160	0.108	0.016	0.557	0.057
Piber	129	0.895	0.104	0.115	0.026	0.585	0.034
Szilvásvárd	53	0.904	0.159	0.104	0.031	0.543	0.043
Topol'čianky	29	1.514	0.217	0.095	0.024	0.557	0.029
Overall	296	1.350	0.078	0.107	0.027	0.566	0.040

Stud means (LS means) for melanoma gradation are obtained from the Model I.

Table 3. Inbreeding and ancestral inbreeding effects on the melanoma (regression slopes[1])

	bf F[1]		bfa F F_a[1]		TIE
Model	**Estimate**	**SE**	**Estimate**	**SE**	**Estimate**
Model I (R^2=0.28)	2.36	2.62			0.30
Model II (R^2=0.30)	-9.00	11.52	19.43	19.19	0.26

The effect of age as well as state stud was highly significant (P<0.001) in both models.
TIE is total inbreeding effect at F = 0.125 and Fa = 0.57 (for the Model II)

Table 4. Heterogeneity of the inbreeding effects (regression slopes[1]) among state studs (Model III, R^2=0.30)

State stud	bf F[1]		TIE
	Estimate	**SE**	**Estimate**
Đakovo	-8.43	6.58	-1.05
Lipica	13.54	10.21	1.69
Piber	3.10	3.95	0.39
Szilvásvárd	8.94	5.21	1.12
Topol'čianky	-10.43	9.06	-1.30

Effect of age and stud was highly significant (P<0.001). TIE is total inbreeding effect at F = 0.125

Table 5. Heterogeneity of the ancestral inbreeding effects (regression slopes[1]) among state studs (Model IV, R^2=0.33)

State stud	bf F[1]		bfa F F_a[1]		TIE
	Estimate	**SE**	**Estimate**	**SE**	**Estimate**
Đakovo	68.81	52.94	-147.70	100.64	-1.92
Lipica	41.70	23.55	-38.38	32.74	2.48
Piber	-19.89	20.35	35.40	31.10	0.03
Szilvásvárd	-45.22*	22.22	103.16*	41.24	1.70
Topol'čianky	-39.95	44.87	56.54	82.37	-0.97

In addition to the significant heterogeneity of the interaction between inbreeding and ancestral inbreeding (*P<0.05) the effect of age and stud was highly significant (P<0.001). TIE is total inbreeding effect at F = 0.125 and Fa = 0.57

If heterogeneous inbreeding effects among state studs are present the estimates based on a common slope (regression coefficient) do not adequately describe a real phenomenon. For that reason, we tested heterogeneity of regression slopes (Model III) as well as heterogeneity of ancestral inbreeding effects (Model IV) among state studs.

Although the estimates varied across studs they were not significantly different (see Table 4).

Weak statistical significance (P=0.049) was obtained for the interaction model with ancestral inbreeding (Table 5). However, estimates from the model with interaction were not consistent and the only significant estimate (Szilvásvárd) was opposite to a purging effect (positive ancestral inbreeding). In addition, the whole model did not yield in much better model fitting and the obtained significance is probably just a statistical artefact.

The results of this analysis suggest that, at the present level, inbreeding does not appear to be a factor that substantially causes melanoma in Lipizzan horses. However, the reader should be aware that it is difficult to estimate inbreeding effects by regression analysis if a small number of genes with large effects is present (Curik et al. 2001). In addition, the presence of epistatic effects might further reduce the precision of the estimation and complicate conclusions that follow from the analysis (Curik et al. 2001). Unfortunately, the genetic architecture of the melanoma inheritance is still unknown. Rieder et al. (2000), using segregation analysis, were not able to reach a conclusion between polygenic, mixed and single locus inheritance of melanoma. Polygenic inheritance (h^2 = 0.36) was also not excluded in the analysis of Seltenhammer (2000). On the other side, possible existence of the genes with large effect (Johnsson and Jackson 1992; Rieder et al. 2000) and epistasis (Johnsson and Jackson 1992) was also suggested.

CONCLUSION

In general, although some indications about the relationship between ancestral inbreeding and melanoma were present, inbreeding does not appear to be a factor that substantially influences the expression of melanoma in Lipizzan horses. In order to understand the genetic background of this disease better, other hypotheses on the inheritance of melanomas will have to be tested.

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