COBISS: 1.08
Agris category code: L10

THE EFFECT OF INBREEDING ON MELANOMA AND VITILIGO OCCURRENCE IN OLD KLADRUBER GREY HORSES [1]

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ABSTRACT

The relationship between inbreeding and melanoma status (graded from 0 to 4) and vitiligo depigmentation (graded from 0 to 3) was analysed by regression model. The study includes 376 animals of both sexes at the age of 1–25 years. The evaluation of horses was performed repeatedly during 4 consecutive years. Melanoma and vitiligo were detected visually and by palpation. The average inbreeding coefficient (FX) calculated from 33 generations of ancestors reached 13 %. Individual inbreeding generally had a significantly negative impact on melanoma and vitiligo in back body parts and non-significant effect on vitiligo in head part. In general, inbreeding appears to be a factor that potentially influences the expression of melanoma and vitiligo in Old Kladruber horses.

Key words: horses, Old Kladruber breed, inbreeding, melanoma, vitiligo

1 INTRODUCTION

Old Kladruber is the only original Czech horse breed, bred especially as a carriage horse for ceremonial purposes. The baroque character of the breed has been conserved until now. This breed is an important gene resource with unique characteristics and high cultural and historical value and has been continually kept in the territory of the Czech Republic for more than four hundred years. The history of the Old Kladruber breed can be divided into several periods. In the first period (till 1914), the Old Kladruber was bred as a ceremonial horse for the Austro-Hungarian Empire. In the second period (till 1938), i.e., in a very short period from the overall aspect, a substantial reduction in the Old Kladruber population size occurred, especially in the black variety. In the third period (till 1973) the regeneration of this breed was realized, there was an increase in genetic variability of the Old Kladruber population due to using individuals from other related breeds. In the fourth period (till 1996), the number of individuals have increased. In the fifth period (since 1996), the Studbook of the Old Kladruber was closed to prevent the influx of unrelated genes. Regarding above mentioned historical development, it is obvious that Old Kladruber is strongly affected by inbreeding (Vostrá-Vydrová *et al.*, 2016). The values of inbreeding describe a change in the genetic structure of a population in favour of the homozygosity of gene sets and at the cost of heterozygosity of the gene pool and implies a loss of genetic variability that may cause phenotypic defects. The inbreeding depression was observed in many traits in different horse breeds – racing performance (Klemetsdal, 1998), morphological traits (Vostrý *et al.*, 2011) and reproductive traits (Van Eldik *et al.*, 2006).

The higher susceptibility of dermal melanoma was recognized in grey horses (Fleury *et al.* 2000). The melanoma is usually noticed in greys at the age of 5–6 years (Jeglum, 1997). Very rarely, cases of the early melanoma stadium were noticed at the age of 3 years (Rodriguez *et al.*, 1997). The places, where melanoma typically oc-

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curs, are the peri-anal and anal region, perineal region, praeputium, and udder. It could be also noticed on lips, eyelids, and ears (Fleury *et al.*, 2000). The thorough characteristics of skin melanoma in grey horses have been unknown yet, supposing it is mainly benign tumour with the possibility of change to malignant stage later on (Solkner *et al.*, 2004). Hereditary effects on melanoma prevalence suggest Futas *et al.* (2012) and Hofmanová *et al.* (2015) in grey Old Kladrubers and Curik *et al.* (2013) in Lipizzan horses. A higher incidence of melanoma at greys is in some papers related to skin depigmentation called vitiligo, which appears on the same body parts where melanoma occurs (Rieder, 1999). The melanoma and vitiligo association was also reported in humans (Lerner and Kirkwood, 1984; Schallreuter *et al.*, 1994). Vitiligo is a depigmenting disorder creating white patches due to the loss of melanocytes in epidermis (Solkner *et al.*, 2004). There is a current incidence of vitiligo in Old Kladruber grey horses without a visible negative influence. In humans vitiligo is the most often considered an autoimunne disorder (Ongenae *et al.*, 2003) possibly related with other diseases (Spritz, 2007). Unfortunately, the genetic architecture of the melanoma and vitiligo inheritance is still unknown. Essien and Harris (2014) mentioned grey horses as valuable animal model for vitiligo studying in humans.

The objective of this study was to analyze the effects of inbreeding on melanoma and vitiligo depigmentation in Old Kladruber grey horses.

2 MATERIAL AND METHODS

The study included a total of 376 grey horses of the Old Kladruber breed aged from 1 to 25 years. Due to a low number, horses older than 21 years were merged to one group for statistical analysis. By repeated measurements in four consecutive years 702 records were obtained (1–4 records from each individual (160 horses were evaluated once, 122 horses twice, 78 horses three times, at 16 horses all four records were obtained). Out of 376 horses, there were 61 % (230) females and 39 % (146) males. Besides the horses bred at the National Stud Kladruby nad Labem, other animals from smaller private breeders have been included to the evaluation as well. The incidence of melanoma was assessed visually and by palpation. The incidence of depigmentation was assessed separately in the head part (vitiligo_F) and back parts – anal and perineal area and external genitals (vitiligo_A). All horses were inspected by a single person. Evaluation was done according to the scale first published by Solkner *et al.* (2004) and in agreement with Curik *et al.* (2013) (Tables 1 and 2). For more details above experimental design see Hofmanová *et al.* (2015).

The total inbreeding coefficient (F_X) of each individual was estimated by a tabular method (Falconer and Mackay, 1996) based on VanRaden's method (1992). The pedigree depth was 33 generations. For more information about the quality of pedigree and other population details see Vostrá-Vydrová *et al.* (2016).

Fixed effects influencing all three traits were analyzed by a generalized linear model using the MIXED procedure of SAS (Statistical Analysis System, Version 9.1, 2004) with using repeated measurements. The statistical models comprised the following effects:

$$melanoma = \mu + b_1 AGE + b_2 AGE^2 + b_3 F_X + e,$$
$$vitiligo\_A = \mu + b_1 AGE + b_3 F_X + e,$$
$$vitiligo\_F = \mu + b_1 AGE + b_2 AGE^2 + b_3 F_X + e,$$

where μ is overall mean, $b_1 AGE$ is fixed linear regression on age at evaluation, $b_2 AGE^2$ is quadratic regression on age at evaluation, $f_3 F_X$ is linear regression on individual coefficient of inbreeding. Other tested effects (sex, farm, year of evaluation) were non-significant.

3 RESULTS AND DISCUSSION

Descriptive statistics for melanoma, vitiligo_A, vitiligo_F and individual inbreeding coefficient are shown in Table 3. Overall mean for melanoma was smaller than mean value observed in study Curik *et al.* (2000), which is probably due to high number of young horses in Old Kladruber dataset. The overall inbreeding level was higher than observed in other horse populations. In other Old Spanish breeds, similar but somewhat lower inbreeding coefficients were reported – 11 % (Zechner *et al.*, 2002), 8 % (Valera *et al.*, 2005) and 11 % (Vicente *et al.*, 2012). The F_X coefficient of 7 % was also estimated in Spanish Arabian horses (Cervantes *et al.*, 2008). High

Table 1: *The occurrence and quantification of melanoma*

Melanoma stage	Description
0	Absence of melanoma
1	Early stages of plaque type or nodules 0.5 cm in diameter
2	Several nodules 0.5 cm in diameter or one nodule 2 cm in diameter
3	One nodule or more 5 cm in diameter, or subcutaneous melanoma
4	Extensive melanoma covered with skin, skin destruction, metastases

Table 2: *The occurrence and quantification of vitiligo_A and vitiligo_F*

Vitiligo stage	Description
0	Total skin pigmentation
1	Small scarcely non-pigmented spots
2	Continuous, larger non-pigmented skin patches
3	Large non-pigmented skin patches

inbreeding and relatedness coefficients in all reference population indicate a loss of genetic variability in the Old Kladruber population.

The effect of individual inbreeding on the analysed traits is shown in Table 4. The estimated effects of individual inbreeding on melanoma and vitiligo_A were positive and significantly different from zero. In contrary estimated effect of individual inbreeding on vitiligo_F was negative and non-significant. These results are consistent with previous findings on different character of vitiligo_A and vitiligo_F in Old Kladruber population (Hofmanová *et al.*, 2015). Individual inbreeding coefficient had numerically greater effect on vitiligo_A than on melanoma status. Although the effect of inbreeding was found statistically significant, low values of estimated regression coefficients (Table 4) indicate minimal changes of melanoma and vitiligo_A grade with increase of inbreeding coefficient (0.16 for melanoma and 0.20 for vitiligo_A by 10 % increase in inbreeding). The results found in Old Kladruber breed do not completely correspond with those obtained by Curik *et al.* (2000), who published that inbreeding does not appear to be a factor that substantially causes melanoma in Lipizzan horses. Gomez-Raya *et al.* (2009) also did not find out inbreeding depression for the number of tumours in Sinclair swine. In humans Birlea *et al.* (2008) reported high incidence of vitiligo in isolated inbred Romanian population. The higher degree of melanoma and vitiligo_A in more inbred individuals could be connected with the higher level of homozygosity including GREY locus (GG genotype). The significant effect of GREY locus genotype on traits studied was previously confirmed by Curik *et al.* (2013). Unfortunately GREY locus genotypes were not available for Old Kladruber population.

Table 3: *Descriptive statistic for melanoma, vitiligo_A, vitiligo_F and individual inbreeding coefficient*

Traits	Mean	SD
melanoma	0.235	0.647
vitiligo_A	0.374	0.698
vitiligo_F	1.035	0.964
F_X	0.129	0.042

Table 4: *Inbreeding and ancestral inbreeding effects on the melanoma (regression slopes)*

Trait	b_3F_X	SE	Significant
melanoma	1.601	0.440	**
vitiligo_A	1.993	0.502	**
vitiligo_F	−0.290	0.789	n.s.

where b_3F_X is linear regression on level of inbreeding, SE – standard error of estimated linear regression, ** is $p < 0.01$, n.s. – non-significant

4 CONCLUSION

This study demonstrates that negative impact of inbreeding is likely to have occurred in the Old Kladruber horse population for melanoma and vitiligo_A, but not for vitiligo_F. Although the statistically significant values of inbreeding effect indicate the possibility of inbreeding depression, the influence on traits studied is minimal from practical point of view. For a thorough analysis of effect of inbreeding depression on melanoma and vitiligo_A, large population with a deep well-recorded pedigree is required. For better understanding of inbreeding effect on melanoma and vitiligo as well as for unravelling the genetic background of these disorders in general, the further research is needed.

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