

Genetic parameters of insect bite hypersensitivity in the Old Grey Kladruber horse¹

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ABSTRACT: The objective of this study was to assess the genetic parameters of insect bite hypersensitivity (IBH) in the Old Grey Kladruber horse, an original Czech warmblood horse breed. Insect bite hypersensitivity is a recurrent allergic skin disease affecting horses worldwide. Its etiology is multifactorial. The defect is genetically controlled, and the starting impulse is a bite by midges of the *Culicoides* spp. and less frequently *Simulium* spp. Knowledge about the associated genes is limited. Horses were kept by the National Stud in Kladruhy (1,146 measurements) and by 10 private breeders (63 measurements). The horses were visually scored by the same inspector over a period of 13 yr from 1996 to 2009. A linear logistic model with a binary variable was used for subsequent statistical analysis. The fixed effects of the farm, year of evaluation, age of evaluation, and sex were significant, and the nonsignificant effect was the intensity of greying. The animals from the National Stud were more strongly affected by IBH than animals from private studs. The National

Stud is located near the Labe River, at an altitude of 206 m; there are bottomland pastures in the vicinity, blind river arms, and numerous pools and marshes, and the climate is mild. These conditions are convenient for horse keeping but also for the propagation of *Culicoides*. The stallions were less affected than the mares. The occurrence in years of age was influenced by selection; scoring began in the first year of age, and when the young animal was repeatedly positive, it was eliminated from breeding. The estimated h^2 differed significantly from 0, and the direct h^2 were 0.626 in the animal model with a maternal effect, 0.359 in the animal model without a maternal effect, and 0.363 in the animal model without correlation between direct and maternal effect. The maternal h^2 was 0.305. The estimated repeatabilities were 0.636 with a maternal effect, 0.615 without a maternal effect, and 0.623 without correlation. The h^2 of IBH was found to be medium to high, so there is a possibility to reduce the prevalence of IBH by selection.

Key words: allergy, Grey Kladruber, heritability, horse, repeatability, summer eczema

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INTRODUCTION

Horses develop many skin and respiratory disorders that are attributed to allergies (Fadok, 2013). The most often studied is insect bite hypersensitivity (IBH) in horses. Insect bite hypersensitivity is a re-

current allergic skin disease and is the most common allergic disease affecting horses worldwide (Schurink et al., 2013). Its etiology is multifactorial. There is a common consensus that the defect is genetically controlled, and the starting impulse is a bite by midges of the *Culicoides* spp. and less frequently *Simulium* spp. The subsequent allergic reaction is a result of hypersensitivity to the allergens contained in their saliva (van der Rijt et al., 2008; Wilson et al., 2008). The main symptoms are pruritus (itchiness), scaling, and hair loss. Afflicted animals could be absolutely unfit for any performance (Wintzer et al., 1999; Sloet van Oldruitenborgh-Oosterbaan et al., 2001). The causative allergens have not been characterized at the

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molecular level, but Schaffartzik et al. (2012) identified some salivary gland proteins as relevant allergens.

The genes contributing to the genetic variance associated with IBH remain unknown despite recent analyses in this area (Schurink et al., 2012, 2013; Shrestha et al., 2015). However, Velie et al. (2016) recently published promising results of a genome-wide scan. They identified 2 regions of interest on chromosome 8 with 6 genes; their results have the potential to be a step forward in unraveling the complex nature of hypersensitive reactions.

As effective treatment with allergen-specific immunotherapy remains elusive (Schaffartzik et al., 2012; Fadok, 2013), genetic improvement is a desirable course of action (Eriksson et al., 2008; Schurink et al., 2009). Hence, the objective of this study was to assess the genetic parameters of IBH in the Old Grey Kladruber horse, which is an original Czech warmblood horse breed and an animal genetic resource. This paper continues a previous study on the breed (Raskova and Citek, 2013).

MATERIAL AND METHODS

Animals

All data were collected under routine and humane horse management programs but were not subject to institutional review.

The Old Grey Kladruber horses were kept by the National Stud in Kladruhy (1,146 measurements on 476 horses) and by 10 private breeders (63 measurements on 18 horses). Due to the small number of individuals in private farms, these were included in the study as 1 subgroup.

The distribution of measurements by sexes over years and over ages is given in Table 1. Of 494 horses, 353 (71%) were always diagnosed healthy and 141 (29%) horses were diagnosed as afflicted 1 or more times. The horses were descendants of 23 stallions and 148 mares. The stallions fathered on average 21.48 foals with a SD of 15.68 foals, a median of 20.00 foals, a minimum of 5, and a maximum of 58. The mares produced on average 3.33 ± 2.63 foals, a median of 3 foals, a minimum of 1, and a maximum of 11. The number of measurements per horse was 2.45 ± 2.88 , with a minimum of 1 and a maximum of 14. The pedigree set for the estimation of genetic parameters comprised 5 generations of ancestors (892 animals).

Data on the Old Grey Kladruber horses were provided by the stud book. The horses were visually scored by the same inspector over a period of 13 yr from 1996 to 2009. The evaluation of the herd occurred twice a year, in May and in October. The diagnosis was made by a veterinarian based on the occurrence of typical skin lesions. The particular characteristic of IBH showed a

binary distribution as follows: 0, negative, indicating the absence of clinical symptoms, and 1, positive, indicating clinical symptoms. When taking into account every assessment, 63% of horses were diagnosed as 0 and 37% of horses were diagnosed as 1. Every assessment was included separately in the evaluation, so the horse was added repeatedly as it was scored consecutively during its life; therefore, the frequency of IBH including the individual horse as healthy or afflicted (71% to 29%) is not equal to the frequency in the whole dataset as measured successively during horse's life.

The intensity of greying was assessed successively for every horse. It was made subjectively according to the extent of greying; typically, full greying is achieved in the breed between the 5th and 8th year of age.

Statistical Analysis

Statistical analyses for the characteristics of IBH data were performed by application of a linear logistic model with a binary response variable, which was modeled as a binomial random variable (y_i). The dependent variable (y_i) can take the value 1 with a probability of π_i being the one of occurrence of a given phenomenon for the characteristics of IBH or the value 0 with a probability of $1 - \pi_i$ being the one of absence in observation i . The logistic model uses a link function $g(\pi_i)$ linking the expected value to the following nonlinear function:

$$y_i = g(\pi) = \log \left[\frac{\pi_i}{1 - \pi_i} \right]$$

where π_i is the probability of IBH being recorded.

The analysis of fixed effects influencing the IBH characteristics was performed with a generalized linear model using SAS GLIMMIX by repeated statements for the purpose of repeated measurements (SAS Inst. Inc., Cary, NC). Included fixed effects were year of evaluation (1996, ..., 2009), age of evaluation (1, ..., 16), sex (stallions or mares, geldings were not included), and farm (National Stud or private). The least squares means of the effects analyzed in the model were estimated on the logit scale and subsequently back transformed to the original scale (probability) using the following inverse link function:

$$\pi_i = \frac{\exp(\hat{y}_i)}{1 + \exp(\hat{y}_i)}$$

where $\hat{y} = X\hat{\beta}$ and $\hat{y}$ is the expected value of IBH, X is the incident matrix of fixed effects, and $\hat{\beta}$ is the vector of estimated parameters of linear predictors applying the least square means statement.

Table 1. Distribution of data by sexes over years and over ages

Year of evaluation	No. of measurements males/females	% males/females	Age, yr	No. of measurements males/females	% males/females
			1	81/98	6.7/8.11
1996	3/13	0.25/1.08	2	74/87	6.12/7.20
1997	6/16	0.5/1.32	3	81/89	6.70/7.36
1998	40/40	3.31/3.31	4	88/71	7.28/5.87
1999	43/42	3.56/3.47	5	63/63	5.21/5.21
2000	41/45	3.39/3.72	6	24/49	1.99/4.05
2001	46/55	3.80/4.55	7	20/44	1.65/3.64
2002	36/53	2.98/4.38	8	14/31	1.16/2.56
2003	42/46	3.47/3.80	9	8/25	0.66/2.07
2004	25/51	2.07/4.22	10	12/27	0.99/2.23
2005	33/48	2.73/3.97	11	9/26	0.74/2.15
2006	36/66	2.98/5.46	12	7/21	0.58/1.74
2007	31/65	2.56/5.38	13	8/21	0.66/1.74
2008	43/60	3.56/4.96	14	6/18	0.50/1.49
2009	74/110	6.12/9.10	15	1/14	0.08/1.16
			16	3/26	0.25/2.15
Total	1,209	100		1,209	100

The variances of genetic and nongenetic effects in the studied trait were estimated by the REML method using the DMU software package (Madsen and Jensen, 2013), which contained the logit link function. Heritabilities were calculated using the variance of the logit link function, which implies a correction of the residual variance by a factor of $\pi^2/3$. The following single-trait animal model for the estimation of genetic parameters was used (model I):

$$\log\left(\frac{\pi_{ijklmno}}{1 - \pi_{ijklmno}}\right) = \text{Year}_i + \text{Age}_j + \text{Sex}_k + \text{Farm}_l + a_m + m_n + pe_o$$

where Year_i is a fixed effect of the i^{th} year of evaluation ($i = 1996, \dots, 2009$), Age_j is a fixed effect of the j^{th} year of the horse ($j = 1, \dots, 16$), Sex_k is a fixed effect of the k^{th} sex ($k = \text{stallion or mare; geldings were not included}$), Farm_l is a fixed effect of the l^{th} farm ($l = \text{National Stud or private}$), a_m is a random direct genetic effect of the m^{th} individual with $\sim N(0, A\sigma_a^2)$, m_n is a random maternal genetic effect of the n^{th} dam with $\sim N(0, A\sigma_m^2)$, pe_o is a random permanent environment of the o^{th} horse with $\sim N(0, I_{pe}\sigma_{pe}^2)$, I_{pe} is the identity matrix of the appropriate dimensions, A is a matrix of the additive genetic relationships among individuals, σ_a^2 is the additive genetic variance of direct genetic effects, σ_m^2 is the additive genetic variance of maternal genetic effects, and σ_{pe}^2 is the permanent environmental variance.

It was assumed that only direct and maternal genetic effects could potentially be correlated, $r_{am} = \sigma_{am} / (\sigma_a \cdot \sigma_m)$, where σ_{am} is the genetic covariance of direct and maternal genetic effects. The DMU package was

also used for the calculation of the SE of estimated genetic and nongenetic variance.

The following population parameters were derived from the estimated variance-covariance components:

$$\sigma_p^2, \text{phenotype variance } (\sigma_p^2 = \sigma_a^2 + \sigma_m^2 + \sigma_{am}^2 + \sigma_{pe}^2 + \sigma_e^2);$$

$$h_a^2, \text{coefficient of direct } h^2 (h_a^2 = \sigma_a^2 / \sigma_p^2);$$

$$h_m^2, \text{coefficient of maternal } h^2 (h_m^2 = \sigma_m^2 / \sigma_p^2);$$

$$r_{am}, \text{genetic correlation of direct and maternal effect } [r_{am} = \sigma_{am} / (\sigma_a \cdot \sigma_m)]; \text{ and}$$

$$R, \text{repeatability } [R = (\sigma_p^2 - \sigma_e^2) / \sigma_p^2].$$

Animal models without the random maternal effect or without correlations between direct and maternal effects were run as well. The phenotypic variance for the animal model without maternal effects was $\sigma_p^2 = \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2$ (model II), and for the animal model without correlations between direct and maternal effect, the model was $\sigma_p^2 = \sigma_a^2 + \sigma_m^2 + \sigma_{pe}^2 + \sigma_e^2$ (model III).

The suitability of the models used for the estimation of genetic parameters of IBH was tested on the basis of the ratio of residual error variance to the total phenotypic variance (e^2 ; Jamrozik et al., 2005) and Akaike information criterion (AIC).

RESULTS AND DISCUSSION

Effects on the Incidence of Insect Bite Hypersensitivity

The fixed effects of farm, year of evaluation, age of evaluation, and sex were significant when evaluating the probability of occurrence of IBH (Table 2). The intensity of greying was not significant.

The geographic location of the horse significantly affected the prevalence and severity of IBH in our study. The horses from the National Stud had a greater risk of being affected by IBH than horses from private studs. The National Stud is located near the Labe River at an altitude of 206 m. There are bottomland pastures in the vicinity, blind river arms, numerous pools and marshes, and the climate is mild. The conditions are convenient for horse keeping but also for the propagation of *Culicoides*. Several studies have shown the effects of the location of the horse on the prevalence of IBH. Namely, climate and habitat factors were of importance, where environments with a low IBH prevalence had high rainfall, many cold days, and few warm days per year (van Grevenhof et al., 2007; Eriksson et al., 2008; van den Boom et al., 2008). These factors are assumed to have an influence on the amount of *Culicoides* to which horses are exposed. Our results support those previous statements.

The most relevant influence on the incidence of IBH was the year. The highest risk of IBH was observed from 2000 to 2002, and the lowest was observed in 1996, 1997, and 2009. The differences may be related to year-to-year climate variation influencing the quantity of *Culicoides*. According to weather records, the years with high IBH showed higher temperatures and slightly higher humidity, both during the entire year and in the summer season from March to September (Czech Hydrometeorological Institute, 2016).

Sex showed statistical significance; in accordance with the previous study of Eriksson et al. (2008), the stallions had less risk to be affected than mares (Lange et al., 2005). The age at evaluation showed significant and sizeable differences in IBH prevalence as well. However, selection influenced the IBH prevalence per age at evaluation. Scoring started after the horse became 1 yr of age. When the young horse was repeatedly diagnosed as positive, it was eliminated from breeding; thus, a declining prevalence of IBH after the 4th year of age was observed. A relatively high occurrence of IBH in young horses may be specific to the breed when the Old Grey Kladruher horse is bred in restricted number, and the gene inflow was rather limited for generations. This may cause the accumulation of undesirable genes, giving rise to IBH at a young age. A moderate rise in the subsequent years of age (with fluctuation) may be ascribed to the long-term effect of the exposure of horses to allergens.

Table 2. Estimates of least square means (LSM; probability of risk of occurrence of IBH [%]) and SE of fixed effects for insect bite hypersensitivity in Old Grey Kladruher horses¹

Effect		LSM	SE	n^2		LSM	SE	n	
Farm	Stud	22.76	2.77	1,146	Sex	Male	7.30	2.48	499
	Private	2.83	1.37	63		Female	9.82	3.05	710
Year of evaluation						1	22.1	6.58	179
	1996	0.96	1.05	16		2	20.5	6.15	161
	1997	0.76	0.32	22		3	21.26	6.21	170
	1998	19.35	6.31	80		4	10.55	3.52	159
	1999	13.09	4.61	85		5	5.13	1.93	126
	2000	30.30	8.46	86		6	3.00	1.34	73
	2001	20.50	6.40	101		7	4.09	1.90	64
	2002	23.89	7.30	89		8	6.01	2.82	45
	2003	15.03	5.16	88		9	5.19	2.32	33
	2004	17.75	5.92	76		10	8.27	3.87	39
	2005	17.42	5.71	81		11	5.48	2.01	35
	2006	13.87	4.65	102		12	5.35	2.03	28
	2007	10.40	3.71	96		13	8.84	4.05	29
	2008	10.27	3.72	103		14	9.56	4.56	24
	2009	0.21	0.10	184		15	7.70	3.65	15
						16	16.61	8.26	29

¹Only significant effects were included into the Table; the nonsignificant effect was the intensity of greying.

²n is the absolute number of measurements. Each horse was included multiple times and shown is how many times it was evaluated consecutively during its life.

Genetic Parameters of Insect Bite Hypersensitivity

Estimates of the variance components, h^2 , and maternal effects for all tested animal models are shown in Table 3. The estimated h^2 for direct effect significantly differed from 0, and the direct h^2 were 0.626 in model I and 0.389 and 0.363 in models II and III. The maternal h^2 was 0.305 for model II and 0.058 for model III; however, maternal h^2 for model III was not significantly different from 0. The exclusion of horses from private farms resulted in only negligible changes in evaluated genetic parameters (data not shown). These results indicate that IBH, based on clinical symptoms, is a medium to high heritable trait in the Old Grey Kladruher horse.

The h^2 estimations in our study for models II and III are comparable with those of other authors (Eriksson et al., 2008; Peeters et al., 2015). However, some authors found lower coefficients around 0.1 to 0.2 (Schurink et al., 2009, 2011). The h^2 estimation for model I was higher than all published h^2 . This may be caused by the same environment in which the majority of horses were kept — 95% of the measurements were made in the National Stud. Also the limited number of horses could increase the h^2 . The breed in analysis is a small population, with the population in the Czech Republic containing approximately 250 mares and 20 to 25 stallions. The breed is closed, and there has been

Table 3. Estimates of genetic parameters of insect bite hypersensitivity of Old Grey Kladruber horse, with SE in parentheses¹

Parameter ²	Model I	Model II	Model III
AIC	-3,697	-3,667	-3,687
σ^2_{pe}	0.359 (1.475)	1.848 (0.841)	1.679 (0.838)
σ^2_m	2.640 (1.751)		0.487 (0.629)
σ_{am}	-2.904 (2.123)		
σ^2_a	5.406 (2.684)	3.174 (1.191)	3.024 (1.285)
σ^2_p	8.641	8.164	8.331
h^2_a	0.626 (0.116)	0.389 (0.089)	0.363 (0.098)
h^2_m	0.305 (0.141)		0.058 (0.071)
r_{am}	-0.769 (0.202)		
r^2	0.636	0.615	0.623
e^2	0.364	0.385	0.377

¹Model I is the animal model with a maternal effect, model II is the animal model without a maternal effect, and model III is the animal model without correlations between direct and maternal effect.

²AIC = Akaike information criterion, σ^2_{pe} = the permanent environmental variance, σ^2_m = the additive genetic variance of maternal genetic effects, σ_{am} = the covariance between direct and maternal additive genetic effects, σ^2_a = the additive genetic variance of direct genetic effects, σ^2_p = the phenotype variance, h^2_a = the coefficient of direct h^2 , h^2_m = the coefficient of maternal h^2 , r_{am} is the genetic correlation of direct and maternal effects, r^2 = the repeatability, and e^2 is ratio of residual variance to phenotypic variance.

little gene flow into the population for at least 200 years. Differences among breeds may play a role as well, and there may be differences in the coefficients due to varied computational approaches.

The estimated repeatabilities ranged between 0.615 for model II and 0.636 for model I. Schurink et al. (2009, 2011) have estimated the repeatabilities at 0.30 and 0.89. In fact, one would expect an even higher repeatability in our study: IBH is a chronic disease, and when the horse is afflicted, positive findings in subsequent years are highly expectable. Moreover, as our horses were scored by the same inspector and kept in the same locality, repeatability should be higher than in groups where status was evaluated by different inspectors and where part of the horses could change locality. The probable reason is that in our analysis, the culling of young IBH-positive horses reduced the repeatability. Dohm (2002) states that lower repeatability than expected can be caused by a large negative correlation between direct and maternal effects, which fits with our model I; additionally, if the model for the decomposition of the trait's phenotypic variance is incomplete, e.g., an important component of variance has been omitted from the model, it fits with our model II, where a maternal effect is missing, and with model III, where correlation is missing.

The negative value of direct-maternal correlation may be related to maternal features, such as transfer of various immunological substances via colostrum; to environmental aspects experienced during early life, such as habitat related to allergen exposure; to management

by breeders, such as feeding or stabling; and to genetic ability. Although the estimates of maternal effect were nonsignificant (models I and III), the results show the influence of maternal effect on the occurrence of IBH. As the genetic correlation between direct and maternal effects was included into model I, the variance of animal permanent environmental effect was markedly decreased. This decline was caused by the increase of the direct and maternal variance. The high negative genetic correlation causes high levels of direct and maternal variances and hence h^2 due to a compensatory effect.

Despite fewer positive values of tested criteria (AIC and e^2), model II without maternal effect should not be rejected definitely in estimating the genetic parameters of IBH. For a thorough analysis of maternal effect and correlations between direct and maternal effects of IBH in the Old Kladruber breed, a large population would be required. However, this requirement is difficult to comply with because of the small number of the breed.

Conclusion

The h^2 of IBH was found to be medium to high in the Old Grey Kladruber horse. Methodically, the maternal effect and correlations between direct and maternal effect should be dissected repeatedly in a more numerous population. The estimated repeatability of IBH was lower than may be expected because the young IBH-positive horses were culled and so could not be recorded repeatedly, when repetitive positive diagnoses would be expected in the case of chronic immune disease, and because a large negative correlation between direct and maternal effect was found. The analysis confirmed the possibility of reducing the prevalence of IBH by selection. However, in the case of the small gene resource with high incidence, this would be a long-term and difficult process.

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