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Incidence of Insect Bite Hypersensitivity in a Small Population of Warmblood Horse Breed in the Czech Republic

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ABSTRACT

Insect bite hypersensitivity (IBH) is a serious equine disease with a complex etiology, affecting many breeds. The availability of a small closed population with a relatively high incidence of IBH (28%) offered the chance to contribute to a better understanding of the etiology of IBH. All 736 Warmblood horses, kept in lowland floodplain meadows in the Czech Republic, were analyzed in the period 1998–2010. The morbidity rate in the offsprings of particular stallions was significantly different; the lowest was 10% and the highest 75%. The assumption that the highest occurrence of healthy offspring would be in healthy parents and vice versa was confirmed. The sex of the offsprings was not significant. To decline the disorder prevalence in this population, stud stallions should be IBH negative; afflicted mares can be tolerated to a certain extent, but their male offspring must never be used as stud. Heredity control is necessary, and stallions with IBH+ offspring should be culled. There is to date no reliable treatment available, and therefore, the development of preventive measures is particularly important. The incidence of IBH could be lowered by prudent breeding, and thus immunotherapy and complex herd management would not be necessary. However, this would be a long-term and complex process.

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1. Introduction

Insect bite hypersensitivity (IBH) of horses is a recurrent allergic skin disease, and is the most common allergic disease affecting a wide range of horse and pony breeds. It is caused by biting midges (*Culicoides* spp., less often *Simulium* spp.) and is a result of hypersensitivity to the allergens contained in their saliva [1,2]. Its main symptom is intensive pruritus (itchiness). Affected horses scratch and bite themselves; horses affected in this way can be completely unfit to ride in summer [3,4]. The main season of incidence is from April to October because of the occurrence of biting midges [5].

There is a consensus among some authors that IBH is a disease with a genetic predisposition. The precise mechanism of inheritance is not known, but the results suggest that it is a polygenic, autosomal recessive mechanism, more frequently conferred by the sire line [6]. The expression of the disease is influenced by more than two genes [3,7–9]. The incidence of IBH is influenced by genetic and environmental factors [10].

Besides the direct influence of the allergens in the saliva of the insects, other environmental factors, such as the health status of the horse, nutrition, and grazing management, should be taken into account [11]. The symptoms of the disorder often fade away completely during the winter season and when contact with biting midges is avoided; however, they return along with the insects next summer. The disease usually appears for the first time at maturity—in the fourth or fifth year of age—although the incidence in foals and young horses is sporadic [3]. Sometimes symptoms may appear in old

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horses after they have been moved to localities with biting midges. The clinical status usually becomes milder with increasing age [12]. No reliable treatment for IBH has been developed yet. Horse breeders can only mitigate the symptoms by corticosteroid salves and management changes to avoid contact with the midges.

The objective of the present study was to analyze the incidence of IBH in a small closed population of Warmblood horses. The population is fully closed for at least 80 years, and the gene flow in past 200 years was minimal. The data were provided by the stud book.

2. Material and Methodology

All horses were kept in one stud farm situated in a temperate climate zone, at an altitude of about 200 m above sea level in the eastern part of Bohemia, Czech Republic. Floodplain meadows, lakes, and wetlands were in proximity to the stud farm. The conditions were very good for horse-keeping, but also favorable for the propagation of biting midges. The biting midges prefer warm humid climate and avoid windy and rainy regions [13]. In central Europe, the most common species of *Culicoides* are *Culicoides nubeculosus* and *Culicoides obsoletus* [1].

The incidence of IBH during the period 1998–2010 was investigated. During the studied period, the population amounted to 736 horses in total, of which 30 were stud stallions, 175 mares, and 604 offsprings (73 offsprings were also counted among the dams or sires). The diagnosis was made by one veterinarian based on the occurrence of the typical skin lesions [8] when other causes were dismissed. The evaluation of the herd occurred twice a year, in May and in October. All horses are periodically diagnosed since birth to at least until maturity (4–5 years), or even longer if they are used in breeding.

The total data on IBH incidence in the offspring of the stallions and mares were obtained from the original database by using the method of contingency tables. In stallions fathering more than 20 offsprings, the frequencies of affected animals in relation to the total number of offsprings were compared using the paired difference test in the StatPlus program [14]. In mares, the limit value of nine offsprings was set.

An additive effect of the morbidity rate of parents on the incidence of IBH in offspring was also examined. The offsprings were divided into four groups according to the incidence of IBH in the parents: (I) both parents healthy, (II) affected dam, healthy sire, (III) healthy dam, affected sire, and (IV) both parents affected. These groups were compared by the paired difference test.

A complementary analysis of the additive effect of the morbidity rate of parents was carried out using Pearson χ^2 test of expected distribution in the Statistica program [15]. The test proposes the distribution of frequencies of the particular groups that could be expected if these groups did not have any influence on the frequency of IBH incidence. The percentage change shows how much the actual distribution differs from the expected distribution. A positive change indicates an increase in frequency in favor of healthy descendants, whereas a negative change is in favor of the affected ones.

The influence of factors mother and father status on the IBH frequency in descendants was evaluated using logistic regression. The equation to estimate the variance was

$$\text{IBH} = \mu + F_i + M_j + e_{ijk}$$

where IBH is the evaluated trait coded as “0” for healthy descendant and “1” for affected descendant; F_i is the healthy status of the father coded as “0” for healthy and “1” for affected; M_j is the healthy status of the mother coded as “0” for healthy and “1” for affected; and e_{ijk} is the residual.

Logistic regression also produces odds ratios (ORs) associated with each predictor value. The odds of an event are defined as the probability of the outcome event occurring divided by the probability of the event not occurring. The OR provides a measure of association between the chosen factor and IBH. Then $\text{OR} = 1$ indicates the lack of association, $\text{OR} > 1$ corresponds with the increasing odds and $\text{OR} < 1$ with the decreasing odds of IBH with the factor.

3. Results

3.1. Sire's Effect

Twelve stallions fathered more than 20 offsprings during the studied period. Of these, 11 stallions were free of clinical symptoms, and only one (stallion 11) was affected.

Obviously, there were significant differences on morbidity rate in the descendants of sires analyzed: the lowest rate was 10.3%, whereas the highest amounted to almost 75% (Table 1). The average morbidity rate of the offsprings of the stallions selected was 28.7%, which approximately corresponded to the average of the entire population, that is, 28%. The morbidity rate of the offspring of stallion 11 was significant. Although afflicted himself, this stallion was repeatedly used for breeding. Naturally, the popular sire disseminated IBH extensively in the population.

A significant difference was also found in stallion 2 who had a relatively high morbidity rate in his offsprings (45.5%), although he was healthy. Stallion 2 produced affected offsprings more frequently with healthy mares than with affected ones. Hence, it is assumed to be a carrier of predisposition to IBH in the population.

3.2. Dam's Effect

Thirteen mares bore more than nine offsprings during the studied period (Table 2). The mares used for breeding were affected by IBH more frequently than stallions because of their low number in the small closed population. The morbidity rate of dams in the all studied population was 24.0% (42 of 175), whereas it was only 10% in sires (3 of 30).

Despite quite a high number of offsprings, some dams (Table 2) had relatively few IBH+ offsprings (mares 3 and 7), although in some cases, they were afflicted themselves (mare 12). In contrast, a healthy dam may have had up to one-half of her offsprings affected (mares 6 and 8).

In general, the descendants of the selected dams are affected more frequently (34.7%) than those of the selected

Table 1

Sires with more than 20 offsprings and morbidity rate of their offsprings

Sires	Healthy Offsprings				Affected Offsprings				Total Offsprings
	Male Offsprings	Female Offsprings	Healthy Offspring	% of Total Offspring	Male Offsprings	Female Offsprings	Total Affected Offsprings	% of Total Offsprings	
Stallion 1	6	10	16	76.19	3	2	5	23.81 ^C	21
Stallion 2	6	6	12	54.55	2	8	10	45.45 ^{ABabcd}	22
Stallion 3	8	11	19	82.61	2	2	4	17.39 ^{Da}	23
Stallion 4	9	10	19	70.37	3	5	8	29.63 ^{Ee}	27
Stallion 5	14	8	22	75.86	2	5	7	24.14 ^F	29
Stallion 6	15	16	31	86.11	2	3	5	13.89 ^{AG}	36
Stallion 7	16	19	35	89.74	2	2	4	10.26 ^{BHef}	39
Stallion 8	13	18	31	79.49	1	7	8	20.51 ^{Ib}	39
Stallion 9	23	12	35	77.78	4	6	10	22.22 ^J	45
Stallion 10	25	14	39	73.58	6	8	14	26.42 ^{Kf}	53
Stallion 11	8	7	15	25.42	26	18	44	74.58 ^{CDEFGHIJKLc}	59
Stallion 12	36	28	64	79.01	4	13	17	20.99 ^{Ld}	81
Total	179	159	338	71.31	57	79	136	28.69	474

Stallion 11 was IBH+; other selected stallions with more than 20 offsprings were free of clinical symptoms.

^{abcde}Stallions with identical letters differ significantly ($P < .05$). (Stallion 2 : stallions 3, 8, 11, 12; stallion 4 : stallion 7; stallion 7 : stallion 10).^{ABCDEFGHIJKL}Stallions with identical letters differ significantly ($P < .01$). (Stallion 11 : stallions 1, 3, 4, 5, 6, 7, 8, 9, 10, 12; stallion 2 : stallions 6, 7).

sires (28.7%), but this may have been caused by the higher morbidity rate of dams used for breeding.

3.3. Analysis of the Effect of Both Parents

Table 3 shows the frequencies of animals in groups I–IV. The data confirm the original assumption that the highest number of healthy offsprings is born to healthy parents (group I) and the highest number of affected offsprings is born to affected parents (group IV). It is interesting that a higher number of IBH+ descendants were born to a healthy mare and affected sire (group III) than vice versa. A significantly increased frequency of incidence was found in groups III and IV in comparison to groups I and II. These results suggest that the incidence of the disease in the population is influenced mainly by the sire, which is also expressed in cases where both parents are affected.

Similar results were obtained by an analysis according to the sex of the offspring (data not shown). Hence, the sex

of the offspring did not have a significant influence on IBH incidence in this study.

The results were subjected to Pearson χ^2 test of expected distribution. Observed and expected distribution of all descendants is shown in Table 4, as well as the results of the same test for male and female descendants, respectively.

A significant difference was found between groups III and IV in all offsprings, both male and female descendants. Results listed in Table 4 (test of female descendants only) also show a low, but not significant, negative effect of the dam (a change of -2.0%). The results document that the common effect of both affected parents is somewhat higher than the separate effects of either affected sire or affected dam, but the difference is not significant.

When male and female descendants were tested separately, higher effects of an affected dam on daughters and of an affected sire on sons were observed; however, these differences were not significant.

Table 2

Dams with more than nine offsprings and morbidity rate of their offspring

Dam	Healthy/ Affected Dam	Healthy Offsprings				Affected Offsprings				Total Offsprings After Dam
		Male Offsprings	Female Offsprings	Total Healthy Offsprings	% of Total Offsprings After Dam	Male Offsprings	Female Offsprings	Total Affected Offsprings in	% of the Total Offsprings After Dam	
Mare 1	h	3	4	7	77.78	0	2	2	22.22 ^a	9
Mare 2	h	4	2	6	66.67	2	1	3	33.33	9
Mare 3	h	5	4	9	90.00	0	1	1	10.00 ^{Aef}	10
Mare 4	h	4	4	8	80.00	2	0	2	20.00 ^b	10
Mare 5	h	2	5	7	70.00	1	2	3	30.00	10
Mare 6	h	2	3	5	50.00	4	1	5	50.00 ^e	10
Mare 7	h	5	4	9	81.82	1	1	2	18.18 ^c	11
Mare 8	h	3	3	6	54.55	3	2	5	45.45	11
Mare 9	a	3	2	5	55.56	1	3	4	44.44	9
Mare 10	a	1	3	4	44.44	2	3	5	55.56 ^f	9
Mare 11	a	1	2	3	33.33	2	4	6	66.67 ^{Aabcd}	9
Mare 12	a	4	4	8	80.00	0	2	2	20.00 ^d	10
Mare 13	a	2	4	6	60.00	2	2	4	40.00	10
Total		39	44	83	65.35	20	24	44	34.65	127

a, affected; h, healthy.

^{abcde}Dams with identical letters differ significantly ($P < .05$). (Dam 11 : dams 1, 4, 7, 12; dam 3 : dams 6, 10).^ADams with identical letters differ significantly ($P < .01$). (Dam 3 : dam 11).

Table 3

The morbidity rates of offsprings in relation to the status of both parents

Group	Healthy Offsprings				Affected Offsprings				Total Offsprings in the Group
	Male Offsprings	Female Offsprings	Total Healthy Offspring	% of the Group	Male Offsprings	Female Offsprings	Total Affected Offsprings	% of the Affected Offsprings in the Group	
I (25 s, 132 d)*	150 ^{AB}	135 ^{AB}	285	80.28 ^{AB}	28	42	70	19.72	355
II (25 s, 42 d)	61 ^{CD}	67 ^{Ca}	128	72.73 ^{CD}	15	33	48	27.27	176
III (3 s, 32 d)	9 ^{AC}	10 ^{Aa}	19	35.85 ^{AC}	20	14	34	64.15	53
IV (3 s, 16 d)	1 ^{BD}	2 ^{BC}	3	15.00 ^{BD}	10	7	17	85.00	20
Total	221	214	435	72.02	73	96	169	27.98	604

s, sire; d, dam.

Groups: (I) both parents healthy; (II) affected dam, healthy sire; (III) healthy dam, affected sire; and (IV) both parents affected.

* 25 sires, 132 dams.

^aGroups with identical letters differ significantly ($P < .05$). Column female offsprings-group II : group III.^AGroups with identical letters differ significantly ($P < .01$). Column % of the group-group I : groups III, IV; group II : groups III, IV. Column male offsprings-group I : groups III, IV; group II : groups III, IV. Column female offsprings-group I : groups III, IV; group II : group IV.

Table 5 gives the estimates of parameters and ORs for factors “father” and “mother”. The OR values show that affected father increases the odds of IBH in descendants by 8.45 compared with a healthy father. The confidence intervals do not include 1, indicating reliability of the evaluation. Because the sample size was greater than 500, the evaluation could be considered sufficient to avoid the overestimate of the OR. The influence of fathers was found substantially higher than of mothers. The selection of sires is more demanding compared with the mares; there were three affected sires only (taking into consideration all stallions of the investigated population, not only the ones with more than 20 offsprings), but with a high frequency of affected offsprings (70%). However, the interaction father to mother was not found statistically significant ($P = .3003$), and the OR confidence interval included 1, hence the interaction could be removed from the model.

4. Discussion

Marti et al. [9] reported the prevalence of 4.6% of IBH in Icelandic horses born in Germany. If both parents were affected, the incidence in their offspring was higher (12.2%) than if only one parent was affected (6.5%) or neither of the parents was affected (2.9%). Eriksson et al. [16] reported the total incidence of 26%–72% for Icelandic horses imported to continental Europe. Such a high prevalence occurs in these horses owing to absence of *Culicoides* in Iceland, and therefore vigorous reaction of their mature immune system to allergen exposure.

In our study, the total incidence was of 28%, in offsprings of both affected parents of 85%, in offsprings of one affected parent of 36%, and in offsprings of both healthy parents of 20%. Sires have a greater influence on the spread of IBH in the investigated population than mares. This was

Table 4

Actual and expected distribution of the offsprings by the groups of parents, differences between actual and expected distribution, for both sexes, and male and female descendants separately

Group	Actual Distribution			Expected Distribution		Residuals	% of Change
	Healthy Offsprings	Affected Offsprings	Total	Healthy Offsprings	Affected Offsprings	Affected Offsprings	
I (25 s, 132 d)*	285	70	355	Descendants of Both Sexes		–29.33	8.26
II (25 s, 42 d)	128	48	176	255.67	99.33	–1.25	0.71
III (3 s, 32 d)	19	34	53	126.75	49.25	19.17	–36.17
IV (3 s, 16 d)	3	17	20	38.17	14.83	11.40	–57.02
Total	435	169	604	14.40	5.60		
				435	169		
I (25 s, 92 d)	150	28	178	Male descendants		–16.20	9.10
II (22 s, 37 d)	61	15	76	133.80	44.20	–3.87	5.09
III (3 s, 21 d)	9	20	29	57.13	18.87	12.80	–44.14
IV (2 s, 9 d)	1	10	11	21.80	7.20	7.27	–66.08
Total	221	73	294	8.27	2.73		
				221	73		
I (22 s, 93 d)	135	42	177	Female descendants		–12.81	7.24
II (22 s, 38 d)	67	33	100	122.19	54.81	2.03	–2.03
III (3 s, 18 d)	10	14	24	69.03	30.97	6.57	–27.37
IV (2 s, 7 d)	2	7	9	16.57	7.43	4.21	–46.81
Total	214	96	310	6.21	2.79		
				214	96		

Significance level $P < .000001$.

Groups: (I) both parents healthy; (II) affected dam, healthy sire; (III) healthy dam, affected sire; and (IV) both parents affected.

* 25 sires, 132 dams.

Table 5Parameter estimates and odds ratios of a logistic regression model^a

Effect	Coefficient Estimate	Odds Ratio	95% Confidence Interval
Intercept	–1.42	—	—
Father	2.13	8.45	4.89, 14.58
Mother	0.48	1.62	1.09, 2.40

^aThe interaction father × mother was not found statistically significant ($P = .3003$) and could be removed from the model.

repeatedly confirmed, and perhaps explained by the fact that sires have more offsprings than mares. In this article, the stallion most frequently used for breeding despite suffering IBH was found to have a great influence. In agreement with our results, other authors [6] believed that the disease is inherited from the sires more frequently.

Some authors have focused on the etiology of IBH, clinical symptoms, analysis of allergens causing the disease, period between exposure to the allergen and onset of IBH, and the possibilities of treatment or mitigation of symptoms [5,9,12,17–19]. However, few studies have been published on how to avoid the outbreak of the disease in the breeding process and select parents properly. The heritability of IBH in Dutch Shetland breeding mares was 0.08 on the binary scale and 0.24 on the underlying continuous scale [10]. The authors believe that the incidence can be effectively decreased by selection. Also, other authors stated the low heritability; to halve the incidence may take even 80 years in certain population [20]. Most recent studies [21] confirmed the genetic etiology revealing variants in the MHC class II region being associated with disease susceptibility.

Our study shows the hereditary etiology of the IBH, but there is no evidence for a simple dominant or recessive mode of inheritance. The mating of affected parents gives the ratio of 3 healthy to 17 affected (Table 3) and shows the dominant pattern (Mendelian ratio: 1:3, when both parents are heterozygotes); however, the statistical testing (χ^2 test) did not confirm it. On the other hand, the mating of unaffected parents produced 20% of affected offsprings, which suggests the recessive mode. The defect could be polygenic, and moreover, the environmental aspect contributes to the complexity of the matter. As with other allergies, it is suspected that the individuals without the gene could develop the disease with intense stimulation, but those with the gene would be the ones to be quickly affected. So, the mode of the inheritance must be clarified. Of course, in the genomic age, the single-nucleotide polymorphism chips could be applied to reveal the gene and to identify the carriers. The newest study [22] detected associations with IBH for 24 single-nucleotide polymorphisms on 12 chromosomes; genome-wide studies on other populations are necessary to identify the causal genes.

5. Conclusion

Our results are in agreement with those of other authors that IBH is a disease with a genetic predisposition. To reduce the disease incidence in the analyzed population, the sires should be absolutely free of IBH. In dams, a certain rate of incidence can be tolerated owing to their low number. However, affected mares must never bear stud stallions. Consequently, progeny testing and the culling of

sires with higher frequency of the affliction in their descendants are necessary. Breeding and reproduction in horses are rather problematic compared with other farm animals. In some breeds, the animals are used in breeding after 5 years of age or even later, and the herd management must reflect this condition. Prudent breeding would produce a lowering of IBH incidence. However, this might be problematic for breeds with a high incidence, small population size, and high inbreeding. So, this would be a long-term and complicated process.

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