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A. Schurink, E. M. van Grevenhof, B. J. Ducro and J. A. M. van Arendonk

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Heritability and repeatability of insect bite hypersensitivity in Dutch Shetland breeding mares¹

A. Schurink,² E. M. van Grevenhof, B. J. Ducro, and J. A. M. van Arendonk

Animal Breeding and Genomics Centre, Wageningen University, PO Box 338,
6700 AH Wageningen, the Netherlands

ABSTRACT: Insect bite hypersensitivity (IBH) is a seasonal recurrent allergic reaction of horses to the bites of certain *Culicoides* spp. and is found throughout the world. The aim of our study was to estimate the heritability and repeatability of IBH in the Dutch Shetland pony population. A total of 7,924 IBH scores on 6,073 mares were collected during foal inspections in 2003, 2005, and 2006. Mares were scored for clinical symptoms of IBH from June until February by 16 inspectors. Of all mares, 74.4% ($n = 4,520$) had a single observation, 20.7% ($n = 1,255$) had 2 observations, and

4.9% ($n = 298$) had 3 observations in different years. The overall mean IBH prevalence was 8.8%. Heritability was 0.08 ($SE = 0.02$) on the observed binary scale and 0.24 ($SE = 0.06$) on the underlying continuous scale. Repeatability was 0.30 ($SE = 0.02$) and indicates that including repeated observations of the clinical symptoms of IBH will improve the accuracy of breeding values for IBH. We conclude that IBH, based on clinical symptoms, is a heritable trait in the Dutch Shetland pony population. Therefore, the IBH prevalence in this population can be decreased by selection.

Key words: heritability, horse, insect bite hypersensitivity, repeatability

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INTRODUCTION

Insect bite hypersensitivity (IBH) is a seasonal recurrent allergic reaction of horses to the bites of certain *Culicoides* spp. (Riek, 1954). The allergic reaction causes intense pruritus, which results in self-inflicted trauma; open wounds and secondary infections may follow. Therefore, the welfare of affected horses is seriously reduced. The commercial value of affected horses is also reduced because of disfiguration (Broström et al., 1987; Fadok and Greiner, 1990). Due to extreme discomfort, affected horses are often unsuitable for riding or showing purposes (Gortel, 1998). There is no currently available effective treatment for or prevention against IBH (Anderson et al., 1996; Friberg and Logas, 1999; Pilsworth and Knottenbelt, 2004).

The etiology of IBH is multifactorial in origin and involves environmental and genetic factors (Lange, 2004; Björnsdóttir et al., 2006; Grandinson et al., 2006). Although warm- and cold-blooded horses of various breeds worldwide are affected (Anderson et al., 1988;

Littlewood, 1998; Steinman et al., 2003), heritabilities of IBH have been estimated only in small populations of Icelandic horses (Unkel et al., 1987; Lange, 2004; Grandinson et al., 2006). Therefore, no heritability estimates in breeds other than Icelandic horses are available in the scientific literature.

Sensitization to an allergen is fundamental for allergy development (Holgate and Polosa, 2008). Initial sensitization occurs at a young age, and subsequent exposure to the otherwise-harmless allergen rouses the allergic reaction (Galli, 2000; Holgate and Polosa, 2008). Once affected, horses develop IBH year after year (Anderson et al., 1991); therefore, high repeatability of allergies should be expected even if no estimates of IBH repeatability are available in the scientific literature.

The aim of our study was to estimate the heritability and repeatability of IBH in Dutch Shetland breeding mares to investigate the potential for breeding against IBH and to gain information on the potential of repeated observations on IBH to increase accuracy of selection. Heritability and repeatability were estimated by analyzing phenotypic observations on clinical symptoms of IBH collected during 3 yr.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not required for our study because the collection of phe-

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²Corresponding author: anouk3.schurink@wur.nl

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Table 1. Data description and observed insect bite hypersensitivity (IBH) prevalence per year and overall

Variable	Year of scoring			Overall
	2003	2005	2006	
Observations, n (%) ¹	3,063 (38.6)	1,948 (24.6)	2,913 (36.8)	7,924
Observed IBH prevalence, %	8.6	9.5	8.5	8.8
Mean age, yr (range)	8.1 (3–24)	7.8 (3–22)	8.2 (3–25)	8.1 (3–25)
Housing system, %				
Partly stabled	ND ²	2.0	2.2	2.1
Kept on pasture	ND	98.0	97.8	97.9
WHC, ³ %				
Mini	31.4	28.9	26.4	29.0
Small	20.6	21.9	22.5	21.6
Middle	23.1	23.7	25.2	24.0
Tall	24.9	25.5	25.9	25.4

¹Percentage of total number of observations.

²ND = no data.

³WHC = withers height category: mini = ≤86 cm; small = 87 to 92 cm; middle = 93 to 98 cm; and tall = 99 to 107 cm.

notypes was a noninvasive method additional to the routine foal inspections.

Data

Data on IBH were collected on Dutch Shetland breeding mares from 58 of 90 (64%) regions across the Netherlands, based on 2-digit postal codes, constituting a representative sample of the Dutch Shetland pony breed. The data were collected during foal inspections in 2003, 2005, and 2006 (Table 1). During foal inspections, newborn foals are judged at home by inspectors for studbook entrance. In 2003, the date of scoring was not registered.

Mares were, on average, 8.1 yr of age (SD = 4.1) and were either kept on pasture or partly stabled (Table 1). Mares were grouped by the Dutch Shetland Pony Studbook into 4 categories according to their height at the withers in centimeters. The categories were mini, ≤86 cm; small, 87 to 92 cm; middle, 93 to 98 cm; and tall, 99 to 107 cm (Table 1).

Trait Definition of IBH

In each year, mares were scored for IBH from June until February by 16 inspectors. The IBH scores from December, January, and February (n = 127) were not used, because clinical symptoms of IBH regress during winter when *Culicoides* spp. are usually absent (Anderson et al., 1988). All inspectors were trained by a veterinarian to score the clinical symptoms of IBH by visual observation of occurrence of lesions on IBH-sensitive locations of the body such as the mane, the base of the tail, and the ventral midline.

The IBH status of mares was scored in 3 categories: 0 = absence of clinical symptoms, 1 = dubious or mild clinical symptoms (hair loss), and 2 = clear clinical symptoms (at least bald spots and possible open wounds). Similar IBH scoring methods were used by other researchers (e.g., Broström et al., 1987; Steinman

et al., 2003). Previous results on a subset of our data indicated that IBH categories 1 and 2 were more similar than IBH categories 0 and 1 (Van Grevenhof et al., 2007). Therefore, IBH categories 1 and 2 were grouped together, and resulting IBH scores were analyzed as a binary trait.

Pedigree Information

For the genetic analysis, available pedigree information over 4 generations was considered. The pedigree information was provided by the Dutch Shetland Pony Studbook. Mares descended from 984 sires and 4,455 dams. In total, 0.1% (n = 8) of the mares were descended from an unknown sire, 0.3% (n = 16) of the mares were descended from an unknown dam, and 0.7% (n = 44) of the mares were descended from both an unknown sire and an unknown dam. Sires had, on average, 8.0 offspring in the data, and dams had, on average, 1.2 offspring in the data.

Statistical Analysis

Tested Fixed Effects. Fixed effects considered in our study were year and month of scoring, housing system, climate and habitat, coat color, withers height category, and age. Year and month of scoring might affect IBH prevalence, because the presence and activity of *Culicoides* spp. can differ between years and months (Anderson et al., 1993). Many researchers (e.g., Anderson et al., 1988; Björnsdóttir et al., 2006) advise housing of IBH-sensitive horses to avoid contact with *Culicoides* spp. as a method of prophylaxis. Housing system was tested but a priori not included in the model, because the effect would undeservedly correct for the high IBH prevalence in horses kept stabled (Van Grevenhof et al., 2007). Van Grevenhof et al. (2007) showed that IBH scores were affected by climate and habitat factors, and defined 2 explanatory index variables. The continuous variable “climate” was based on information regarding

annual number of cold and warm days and annual rainfall. The class variable “habitat” was based on information regarding soil type and vegetation. For more details about climate and habitat, see Van Grevenhof et al. (2007). The findings of Van Grevenhof et al. (2007) confirmed their expectations, because *Culicoides* spp. prefer certain weather (Meiswinkel et al., 2000), and an association between IBH prevalence and any particular biotype was suggested by Broström et al. (1987). Horses with dark coat color were more often affected by IBH (Braverman et al., 1983; Lange, 2004). Therefore, the attractiveness of horses with certain coat colors to *Culicoides* spp. could differ (Braverman et al., 1983). Withers height category was considered, because breeding in the Dutch Shetland pony population is practiced within the categories. Age of onset of clinical symptoms of IBH varies widely and there is no fixed threshold age after which the development of clinical symptoms is no longer possible (Anderson et al., 1988; Halldórsdóttir and Larsen, 1991). Some researchers (e.g., Broström et al., 1987; Halldórsdóttir and Larsen, 1991) have shown that IBH prevalence increases with the age of the horse. This does not necessarily indicate an increased susceptibility to IBH in older horses because of age per se, but is thought to be a reflection of an increased risk of exposure to predisposing factors (Halldórsdóttir and Larsen, 1991).

Statistical Model. Fixed effects were established using GLM (SAS Institute, Cary, NC). A linear repeatability animal model was used to estimate variance components, heritability, and repeatability of IBH, using the ASREML program (Gilmour et al., 2006). The model (model 1) was

$$y_{ijklm} = \mu + \text{yearmonth}_i + b_1 \times \text{climate}_m + \text{habitat}_j + \text{WHC}_k + \text{age}_l + \text{animal}_m + \text{pe}_m + e_{ijklm}, \quad [1]$$

where y_{ijklm} = the IBH score (0 or 1); μ = the population mean; yearmonth_i = the fixed combined class effect of year and month of scoring ($i = 1, 2, 3, \dots, 15$); b_1 = the regression coefficient of the continuous covariable of climate (climate_m); habitat_j = the fixed class effect of habitat ($j = 1, 2, 3, \dots, 9$); WHC_k = the fixed class effect of withers height category ($k = 1, 2, 3, 4$); age_l = the fixed class effect of age in years ($l = 3, 4, 5, \dots, 25$); animal_m = the random genetic effect of the m th mare ($m = 1, 2, 3, \dots, 6,073$) with $\sim N(0, \mathbf{A}\sigma_a^2)$; pe_m = the random permanent environmental effect of the m th mare ($m = 1, 2, 3, \dots, 6,073$) with $\sim N(0, \mathbf{I}_{\text{pe}}\sigma_{\text{pe}}^2)$, and e_{ijklm} = the random residual error with $\sim N(0, \mathbf{I}_e\sigma_e^2)$; $\mathbf{I}_{\text{pe}}$ and $\mathbf{I}_e$ are identity matrices of the appropriate dimensions, and $\mathbf{A}$ is a matrix of additive genetic relationships among all Shetland ponies in our study (e.g., Lynch and Walsh, 1998).

The model was extended with a fixed class effect of inspector (model 2) to evaluate the influence of this additional effect on heritability estimates. Model 2 was

$$y_{ijklmn} = \mu + \text{yearmonth}_i + b_1 \times \text{climate}_n + \text{habitat}_j + \text{WHC}_k + \text{age}_l + \text{inspector}_m + \text{animal}_n + \text{pe}_n + e_{ijklmn}, \quad [2]$$

where inspector_m = the fixed class effect of inspector ($m = 1, 2, 3, \dots, 16$), and other components are as described previously.

Heritability and Repeatability

The heritability on the observed binary (IBH positive or negative) scale (h_{obs}^2) was

$$h_{\text{obs}}^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{\text{pe}}^2 + \sigma_e^2},$$

where σ_a^2 = the additive genetic variance, σ_{pe}^2 = the permanent environmental variance, and σ_e^2 = the error variance. Permanent environmental factors are those factors that cause similarity in repeated observations on the same mare. The additive genetic variance, permanent environmental variance, and error variance here together constituted the total phenotypic variance.

The heritability on the observed binary scale was transformed to an underlying continuous scale according to Dempster and Lerner (1950):

$$h_{\text{und}}^2 = h_{\text{obs}}^2 \frac{[p(1-p)]}{z^2},$$

where h_{und}^2 = the heritability on the underlying continuous scale, h_{obs}^2 = the heritability on the observed binary scale, p = the fraction of affected mares ($p = 0.088$), and z = the ordinate of a standard normal distribution at the threshold point corresponding to the fraction p . The heritability on the underlying scale can be interpreted as the heritability of the genetic predisposition to IBH presuming a continuous scale.

The repeatability (r) is the correlation between repeated observations within a mare; that is,

$$r = \frac{\sigma_a^2 + \sigma_{\text{pe}}^2}{\sigma_a^2 + \sigma_{\text{pe}}^2 + \sigma_e^2},$$

where all variance components are as described previously.

RESULTS AND DISCUSSION

A total of 7,924 IBH scores from 6,073 mares were analyzed. Of all mares, 74.4% ($n = 4,520$) had a single observation, 20.7% ($n = 1,255$) had 2 observations, and 4.9% ($n = 298$) had 3 observations in different years. In total, 694 of the 7,924 IBH scores indicated the presence of clinical symptoms; the overall mean IBH prevalence was 8.8%. The IBH prevalence was greatest in 2005 and least in 2006 (Table 1). The IBH status of 34.2% of the dams ($n = 1,525$) was known, because these dams were present as mares in the data. The observed IBH prevalence in mares descending from affected dams was greater (13.4%) than the prevalence in mares descending from unaffected dams (7.7%). The observed IBH prevalence of progeny groups per sire varied from 0 to 37%.

Choice of Statistical Model

Preliminary results showed that housing system ($P = 0.94$) and coat color ($P = 0.56$) were not significant. The effect of the month in which IBH was scored differed among years; therefore, a combined effect of year and month of scoring was included in the model ($P = 0.01$). Prevalence of IBH tended to increase slightly with age ($P = 0.11$).

From the literature it is known that there are regional differences in presence and activity of *Culicoides* spp. (e.g., Riek, 1954; Broström et al., 1987; Grandinson et al., 2006; Van Grevenhof et al., 2007). To correct for regional environmental effects that affect the presence and activity of *Culicoides* spp., climate ($P = 0.005$) and habitat ($P < 0.0001$) were included in the model instead of region based on the 2-digit postal codes in the Netherlands. Region not only expressed regional environmental effects, but also, in part, differences among inspectors, because inspectors scored regionally. Therefore, inspector and region were partly confounded.

Withers height category was highly significant ($P < 0.0001$). Observed IBH prevalence in mares from the mini withers height category was 5.6%, from the small category was 7.0%, from the middle category was 9.9%, and from the tall category was 10.5%. These differences in prevalence might be caused in part by genetic differences among withers height categories, because breeding is practiced within withers height categories. Excluding withers height category from the model could result in overestimation of heritability, and, thereby, overestimation of genetic gain within the Shetland pony population. However, the heritability estimate was equal (data not shown) when withers height category was excluded from the model.

Heritability

The heritabilities of IBH estimated with the applied models were 0.08 (model 1, excluding the inspector) and 0.05 (model 2, including the inspector as a fixed effect) on the observed binary scale, and 0.24 (model 1)

and 0.17 (model 2) on the underlying continuous scale (Table 2). Estimated heritabilities indicate that IBH, based on clinical symptoms, is a heritable trait in the Dutch Shetland pony population. Including inspector as a fixed effect in the model resulted in a decreased heritability estimate (0.05), because the additive genetic variance decreased 33% when compared with the additive genetic variance estimated with model 1 (Table 2). Including inspector as a random effect in the model resulted in an equal heritability estimate (0.05, $SE = 0.02$). The decrease in additive genetic variance might be caused by the fact that inspectors scored regionally. Shetland pony breeding in the Netherlands is regionally organized, primarily because no artificial insemination is used. Therefore, the distribution of Shetland pony families has a regional character, such that different inspectors probably score different Shetland pony families. The partial confounding of inspectors with Shetland pony families could therefore have resulted in underestimation of the heritability when inspector was included in the model. We consider that model 1 (excluding the inspector) provides a better estimate of the heritability. To avoid a genetic effect related to inspectors, inspectors should score mares throughout the Netherlands during future data collection.

The estimated heritability in our study (0.08) is less than the heritability reported by other researchers. This might be because of differences in the observed IBH prevalence (Unkel et al., 1987; Lange, 2004), breed differences (Unkel et al., 1987; Lange, 2004; Grandinson et al., 2006), differences in analytical methods (Unkel et al., 1987), and differences in how the clinical symptoms of IBH are scored (Unkel et al., 1987; Lange, 2004; Grandinson et al., 2006). The clinical symptoms of IBH were scored in various ways, but heritability of binary IBH scores did not differ from heritability of IBH scores on a 0-1-2 scale in our study (data not shown). In Lange (2004), heritability of binary IBH scores (0.36, $SE = 0.08$) hardly differed from heritability of more detailed IBH scores (0.34, $SE = 0.09$) that were made by weighing and averaging all scores (0 to 4) given to 9 clinical symptoms of IBH on 7 locations of a horse. Grandinson et al. (2006) estimated a heritability of 0.26 based on 4 categories; classifying IBH into 3 or 2 categories resulted in decreased heritabilities (0.12 to 0.15). However, it is unknown whether these heritabilities indeed differ, because Grandinson et al. (2006) did not report standard errors. The results suggest that scoring IBH as the absence or presence of clinical symptoms is sufficient for genetic analysis of IBH. However, more accurate heritability estimates might be expected when better methods of scoring IBH become available to detect affected or even sensitized horses on a continuous scale independent of the presence of *Culicoides* spp.

Repeatability

The estimated repeatability was 0.30 (Table 2) and indicates an upper limit to the heritability of IBH in the

Table 2. Additive genetic variance (σ_a^2), permanent environmental variance (σ_{pe}^2), error variance (σ_e^2), and phenotypic variance (σ_p^2), along with heritability on the observed binary scale (h_{obs}^2), heritability on the underlying continuous scale (h_{und}^2), and repeatability (r) of insect bite hypersensitivity

Parameter	Estimate ¹	
	Model 1	Model 2
Variance		
σ_a^2	0.006	0.004
σ_{pe}^2	0.017	0.017
σ_e^2	0.055	0.055
σ_p^2 (SE)	0.078 (0.001)	0.076 (0.001)
Heritability		
h_{obs}^2 (SE)	0.08 (0.02)	0.05 (0.02)
h_{und}^2 (SE_{und}) ²	0.24 (0.06)	0.17 (0.05)
Repeatability		
r (SE)	0.30 (0.02)	0.28 (0.02)

¹Model 1 = the model excluding a fixed class effect of the inspector; model 2 = the model including a fixed class effect of the inspector.

$$^2 SE_{und} = SE_{obs} \frac{[p(1-p)]}{z^2}; \text{ SE after transformation according to Dempster and Lerner (1950).}$$

Dutch Shetland pony population, because repeatability expresses the proportion of variance that is due to permanent environmental and genetic differences among individuals (Falconer and Mackay, 1996). Repeatability also indicates that including repeated observations of IBH clinical symptoms will improve the accuracy of breeding values for IBH.

The estimated repeatability was less than expected; only 27.2% of the mares with IBH clinical symptoms during the first observation also showed clinical symptoms during the second observation (Table 3). This percentage was expected to be greater, because sensitization to an allergen develops at a young age (Holgate and Polosa, 2008), and once affected, IBH is considered to be permanent (Anderson et al., 1991). Clinical symptoms of IBH were present during the first observation and absent during the second observation, possibly because of yearly and monthly fluctuations in environmental factors related to the presence and activity of *Culicoides* spp. (Anderson et al., 1993). Within regions, locally large differences in IBH prevalence may arise, in part because *Culicoides* spp. are weak fliers (Braverman, 1988; Gortel, 1998), and, therefore, stay within a certain distance of the place where they were born. Unfortunately, month of scoring during the first and second observation was only known for 16 mares, because the date of scoring was not registered in 2003. To be certain that mares have been exposed to *Culicoides* spp., and, therefore, show clinical symptoms before they are scored for IBH, the Dutch Shetland Pony Studbook is advised not to score mares early in the *Culicoides* spp. season. Clinical symptoms of IBH were present during the first observation and absent during the second observation, in part because of the changing of environments caused by the sale of mares (9.5%) with repeated

observations. None of the mares that showed clinical symptoms during the first observation and were moved subsequently showed clinical symptoms during the second observation (data not shown). These mares were probably sensitized, but were moved to an environment where *Culicoides* spp. are only occasionally present or even absent (Van Grevenhof et al., 2007), and, thus, did not show clinical symptoms during the second observation. Intervention by owners, such as stabling mares or using eczema blankets to avoid contact with *Culicoides* spp., is of minor importance because most Shetland mares (97.9%) are kept on pasture (Table 1), and eczema blankets for Shetland ponies have only recently become available.

IBH Scoring Method

Scoring of mares by inspectors based on clinical symptoms may not be the most accurate method, but scoring of clinical symptoms is still the gold standard for detecting IBH (Baselgia et al., 2006), and allows inspectors to score a large number of mares. Because inspectors were trained by a veterinarian to score clinical symptoms of IBH, it is reasonable to assume that positive scores indicate IBH. However, other causes of pruritus resulting in similar clinical symptoms cannot be ruled out entirely.

One of the limiting conditions was to develop an IBH scoring method that could be implemented in the routine inspection system of the Dutch Shetland Pony Studbook. Foal inspections seemed most appropriate with respect to preselection. Although only foals are inspected during foal inspections at home, mares (dams) should be present, whereas during organized mare inspections, the mares themselves are inspected. There-

Table 3. Comparison of the absence or presence of insect bite hypersensitivity clinical symptoms during the first and second observations for mares with repeated observations in percentage and number of mares (in parentheses)

Clinical symptoms: first observation	Clinical symptoms: second observation		
	Absent	Present	Total
Absent	94.8 (1,374)	5.2 (76)	100 (1,450)
Present	72.8 (75)	27.2 (28)	100 (103)

fore, there is less preselection when mares are scored for IBH during foal inspections compared with mares being scored at mare inspections.

As a consequence of scoring IBH during foal inspections, geldings and stallions were not considered. Most of the breeding stallions are kept stabled, and, therefore, are less exposed to *Culicoides* spp. Attractiveness of stallions, geldings, and mares to *Culicoides* spp. could differ, but from the literature it appears that sex differences are not to be expected (e.g., Halldórsdóttir and Larsen, 1991; Björnsdóttir et al., 2006). Braverman et al. (1983) and Lange (2004) found a significant effect of sex on IBH prevalence, but Braverman et al. (1983) concluded that stallions were more often affected, whereas Lange (2004) concluded that mares were more often affected. In contrast, many researchers (e.g., Anderson et al., 1988; Halldórsdóttir and Larsen, 1991; Steinman et al., 2003; Björnsdóttir et al., 2006) did not find a significant effect of sex on IBH prevalence.

Selection Effect

A selection effect concerning IBH was found in the data and illustrates the possibility of selection against IBH. The IBH prevalence in mares with a single observation (10.3%) was significantly greater than the prevalence in mares with 2 (7.3%) and 3 observations (5.1%). The number of observations per mare indicates the number of offspring a mare had during the time span of the study, because mares were scored during foal inspections. Mares with 2 or 3 observations are likely to be used frequently for breeding. Therefore, Dutch Shetland breeding mares more frequently used for breeding have decreased IBH prevalences.

As a result of this selection effect, the data set probably contained fewer affected Shetland ponies compared with the number affected in the entire population. This could have resulted in a slight underestimation of the heritability and repeatability of IBH in the Dutch Shetland pony population.

Conclusions and Implications

Based on clinical symptoms, IBH is a heritable trait in the Dutch Shetland pony population. Therefore, the IBH prevalence in this population can be decreased by selection. The estimated repeatability indicates that including repeated observations of the clinical symptoms

of IBH will improve the accuracy of breeding values for IBH.

The IBH scoring method used could be implemented in the routine inspection system of a studbook. To be certain that horses have been exposed to *Culicoides* spp., and therefore show clinical symptoms, it is advised not to score horses early in the *Culicoides* spp. season. However, more accurate or even greater heritability estimates might be expected when better methods other than subjective scoring of IBH become available to detect affected or sensitized horses on a continuous scale independent of the presence of *Culicoides* spp. It seems useful, therefore, to develop methods for early and accurate diagnosis of IBH and to identify genomic regions involved in IBH sensitivity to efficiently select against horses susceptible for IBH. Selection against IBH will decrease economic losses for breeders due to disfiguration of their horses and possible veterinary costs, and, moreover, will increase animal welfare.

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