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Evaluation of potential effects and genetic parameters in conformational limb defects in Pura Raza Española horses

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ABSTRACT

Morphological limb defects are the most frequent defects in horses. The aim of this study was to establish the prevalence, associated effects and genetic parameters of hock and knee defects in Pura Raza Española horses, using two different approaches. In Approach_1, the hock defects analysed were closed, open, convergent and divergent and the knee defects studied were buck, calf, bench and knock. Defects were classified into 3 levels: 0 no defect, 1 slight defect and 2 serious defects. Approach_2, which used a linear scale, in a pair of opposing defects, divided into 5 levels, from -2 to 2, where 0 corresponded to the absence of defects: Lateral view (close/d/open) and rear view (convergent/divergent) hock defect, lateral view (buck/calf) and frontal view (bench/knock) knee defect. A total of 43,358 horses, with an average age of 5.07 years, were evaluated, with a prevalence of horses affected ranging from 3.31% (bench) to 74.12% (convergent). Genetic parameters were estimated using a Bayesian procedure with the BLUPF90 software. Heritability in Approach_1, ranged from 0.25 (bench) to 0.42 (divergent) and in Approach_2, from 0.18 (bench/knock) to 0.24 (convergent/divergent). The opposing defects may be related to different genes, and it is therefore better to study them as separate defects and not on the same linear scale. The highest positive genetic correlation was between calf vs knock (0.70). Our results imply that selection against limb defects is possible and would allow us to reduce the genetic risk of the horses' offspring suffering from them.

HIGHLIGHTS

- There high prevalence of hock and knee defects in PRE horses make the animals unsuitable for sport and cause a significant economic loss to the breeding herds.
- The evaluation of limb defects independently instead of the traditional linear scale, where the opposite defects are located at the extremes, has good results for its genetic evaluation.
- Using the hock and knee defects as a selection criteria in the PRE' s breeding program could contribute to the eradication of major conformation defects which make the animals unsuitable for sport.

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Introduction

The need for accurate examination of morpho-skeletal characteristics in horse populations and their relationship to functionality is a topic of growing interest in most horse breeds (Komosa et al. 2013). Horse conformation is considered a reliable indicator of horse performance and plays an important role when breeding and buying animals for competitions because it correlates with both the quality of their gaits and movement and the general health of the animal

(Welsh et al. 2013; Sánchez-Guerrero, Negro, et al. 2019; Sánchez-Guerrero, Ramos, et al. 2019). The morphological traits included in breeding programs, in addition to allowing us to subject them to an accurate phenotypical analysis at an early age, should show good correlations with the performance of the horses in competitions. In fact, selection for suitable morphological qualities for performance in a sport discipline benefits genetic progress by allowing us to pre-select animals early, even before they begin competing in sports competitions (Ducro et al. 2009).

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Despite the high number of heritable disorders affecting horses (Nicolas 2000; Morgan et al. 2015), there has been little research into specific heritable disorders and what external factors may affect their presentation. This is due to the fact that in horses, the task of identifying diseases or conformation defects of genetic origin can be difficult by the dispersal of foals after weaning, due to the sale, or the existence of many late-onset diseases (Finno et al. 2009). Diseases and limb conformational defects constitute the major part of these disorders, and they have a significant impact on performance and thus severely affect the use of horses in equestrian sports, which in turn affects the profits and economic profitability of the stud farm (Hilla and Distl 2014). They are often caused by underlying complex genetic effects, which may be breed-specific or common in certain types of horses.

The Pura Raza Española (PRE) horses has its origins in Baroque horses and has contributed to the formation of many other equine breeds both in the American continent and in Europe, such as Lusitano, Friesian, Kaldruby (Vicente et al. 2014; Savelkoul 2015; Vostrá-Vydrová et al. 2016). It is an autochthonous foster breed with a current census of 268,425 horses, spread over 66 different countries (MAPA 2022). Incidentally, it is also the oldest equine breed in Spain, with a studbook created in 1912 and currently managed by the Real Asociación Nacional de Criadores de Caballos de Pura Raza Española (ANCCE), which has managed its breeding program since 2003.

To improve both the management and breeding selection of PRE horses, it is crucial to understand better how the different environmental factors contribute to the development of hock and knee defects, with the objective of reducing its prevalence or even eradicate it from the coming generations. The specific aims of this work were: (1) to calculate the prevalence of different hock and knee defects in a significant population of PRE horses; (2) to determine the genetic and non-genetic factors that may be associated with the development of the defects; (3) to estimate the heritability and genetic correlations with two different approaches and compare them. In Approach_1, eight conformational limb defects were analysed independently, while in Approach_2, a linear scale was used with a pair of opposing defects, following the traditional method for genetic evaluations in linear morphological qualification; and (4) to determine the degree of coincidence in the horses, according to their estimated breeding value (EBV), depending on the approach used.

Materials and methods

Description of traits and database

The database included the records of limb conformational defects in 43,358 PRE horses (14,331 stallions and 29,027 females), ranging between 40,607 in knock-kneed defect and 13,286 in divergent hock defect, with a mean age of 5.07 years old. These records were taken between 2012 and 2021, during the morphological evaluation undergone by all PRE horses once in their lifetime before being registered in the main section of the PRE herd book (Sánchez-Guerrero et al. 2016). The evaluations were carried out by some of the 12 specially-trained veterinarians who systematically carry out the basic aptitude tests in this breed (Sánchez, Gómez, Molina, et al. 2013). The phenotypic evaluation of conformational defects was performed while the horse was standing on a hard surface on flat ground, assuming a natural position. The horses were positioned with the forelegs and hind feet parallel, and as close as possible to the perpendicular, with the hooves aligned. No sedatives were used for the evaluation.

To determine the conformational limb defects and their degree or level of expression, the so-called lines of aplomb were used. Lines of aplomb are imaginary vertical lines that start in the forelimbs, from the outer edge of the shoulder joint and the humeroulnar joint, and finish at the ground surface. In the hind limbs, the lines of the aplomb start from the highest point of the buttock and the coxofemoral joint and extend to the ground surface.

Two approaches were used in this study:

- Approach_1: the defects were analysed independently. Lateral view hock defect was divided into 'closed hock defect' and 'open hock defect' and rear view hock defect into 'convergent hock defect' and 'divergent hock defect'. Lateral view knee defect was divided into 'buck defect' and 'calf defect' and frontal view knee defect into 'bench defect' and 'knock defect'. In total, eight conformational limb defects were studied, which were recorded on a linear scale from 0 to 2. Class 0 corresponds to the absence of the defect; class 1 corresponds to the slight presence of the defect and class 2 is the most pronounced degree of defect.
- Approach_2: a linear scale was used in which a pair of opposing defects was included, following the traditional evaluation method in linear morphological qualification (Stock and Distl 2006; Sánchez, Gómez, Molina, et al. 2013; Duensing et al. 2014;

Vicente et al. 2014; Viklund and Eriksson 2018; Perdomo-González et al. 2022). The defects studied were lateral view hock defect ('closed defect' to 'open defect'), rear view hock defect ('convergent defect' to 'divergent defect'), lateral view knee defect ('buck defect' to 'calf defect'), and frontal view knee defect ('bench defect' to 'knock defect'). The study of conformational limb defects was carried out on a linear scale of 5 classes, from -2 to 2. Classes -2 and 2 were the most pronounced degree of defects, classes -1 and 1 corresponded to the slight presence of the defects and class 0 to the absence of defect.

The conformational limb defects studied (Figure 1) were:

Defects related to the direction of the hock in its rear view:

- Closed hock defect. A horse has this defect when the leg forms a very closed angle with the shank.

- Open hock defect. A horse has this defect when the leg forms a very open angle with the shank.

Defects related to the angle of the hock in its rear view:

- Convergent hock defect. When a horse's hocks slant inwards from the line of aplomb.
- Divergent hock defect. When a horse's hocks slant outwards from the line of aplomb.

Defects related to the angle of the knee in lateral view:

- Buck defect. A horse has this defect when the foreleg arches forward. The axis of the foreleg and the shank form a broken line, with the knee in front of the line of the aplomb.
- Calf defect. A horse has this defect when the forelimb arches backwards. A broken line is formed behind the line of poise, between the axis of the forearm and the shank with the knee.

Defects conformational hock	Class		Defects conformational Knee	Class	
	0 (no defects)	2		0 (no defects)	2
Closed			Buck		
Open			Calf		
Convergent			Bench		
Divergent			Knock		

Figure 1. Conformational limb defects in Pura Raza Española horses. Lines of aplomb in the colour red.

Defects related to the angle of the knee in its frontal view:

- Bench defect. A horse has this defect when the knees deviate outwards from the line of aplomb.
- Knock defect. A horse has this defect when the knees deviate inwards from the line of aplomb.

The effects analysed were: gender (2 levels; male and female), age (2 levels; young <4 years old and adult ≥4 years old), coat colour (4 levels; bay, chestnut, black and grey), geographical area (4 levels; Spain, rest of Europe, South + Central America, North America + Mexico), birth stud size (3 levels; <3 foals born/year, 3 to 9 foals born/year and > 9 foals born/year), percentage of classical inbreeding coefficient (4 levels; <3.125%, ≥3.125% to <6.25%, ≥6.25% to <12.50%, ≥12.50%), proportionality index (3 levels; <98, 98–100, >100). The classical inbreeding coefficient according to Wright (1922), which is defined as the probability that the two alleles at any locus in an individual are identical by descent, was computed using Endog software (Gutiérrez and Goyache 2005). The proportionality index was defined as height at the withers * 100 divided by the scapular-ischial length (distance of the straight segment from the union of the scapular-humerus joint to the point of the buttock).

Statistic and genetic analysis

The test used to compute the significance level for the difference between Class 0 and Class 1 + 2 was:

$$|z| = \sqrt{[(N1 * N2)/(N1 + N2)] * |p1 - p2| / \sqrt{p * q}}$$

where: Pr1: Proportion of the first sample; Pr2: Proportion of the second sample; N1: Sample size of the first sample; N2: Sample size of the second sample.

The *p*-value is computed based on the *z*-value for the respective comparison.

A multivariate Generalised Non-linear Model (GLZ), with a multinomial logit distribution, was used to examine associations between the conformational limb defects and the potential effects. An association frequency analysis of all the effects (Maximum-Likelihood Chi-square) was also carried out. Statistical analyses were performed using Statistica 11 for Windows software (StatSoft Inc 2007).

For the genetic evaluations, the genealogical information provided by ANCCE was used. A minimum of 4 known generations of each animal in performance

control were included. Finally, the pedigree file had a total of 92,997 individuals. Genetic parameters were estimated using Blupf90 software (Misztal et al. 2016). A Bayesian approach with threshold models was applied using THRGIBBS3F90. A total of 100,000 interactions were performed, with the first 20,000 considered as burn-in, after which every 100th sample was saved for later analysis. Convergence was checked by visual inspection of the chains. Then, a post-Gibbs analysis was performed using POSTGIBBSf90 (Tsuruta and Misztal 2006) to calculate posterior means, additive and residual variances, and the standard deviations of both approaches.

For both approaches, a univariate model was applied to study the heritability of the conformational limb defects, while bivariate mixed models were applied to study the genetic correlation between the conformational limb defects. For Approach_1, the following variables were included: (1) For hock defects: closed vs convergent, closed vs divergent, open vs convergent, open vs divergent; (2) For knee defects: buck vs bench, buck vs knock, calf vs bench and calf vs knock; (3) For hock-knee defects: closed vs buck, closed vs bench, closed vs calf, closed vs knock, open vs buck, open vs bench, open vs calf, open vs knock, convergent vs buck, convergent vs bench, convergent vs calf, convergent vs knock, divergent vs buck, divergent vs bench, divergent vs calf, divergent vs knock.

The equation in matrix notation used to solve the mixed model was:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

$$\text{with : } \mathbf{u} \sim N(0, \mathbf{A} \sigma_u^2), \mathbf{e} \sim N(0, \mathbf{I} \sigma_e^2)$$

where \mathbf{y} is the vector of observations, \mathbf{X} is the incidence matrix of fixed effects, \mathbf{Z} is the incidence matrix of animal genetic effects, \mathbf{b} is the vector of fixed effects, \mathbf{u} is the vector of direct animal genetic effects, \mathbf{e} is the vector of residuals, σ_u^2 is the direct genetic variance, σ_e^2 is the residual variance, \mathbf{I} is an identity matrix and \mathbf{A} is the numerator relationship matrix. The estimated breeding values (EBV) were estimated using the two approaches. Reliability was calculated as $1 - (PEV^2 / \sigma_u^2)$, where PEV is the prediction error of variance.

Results and discussion

The PRE breeding program includes as its main selection criteria the improvement of conformation and parameters related to sporting performance. While many parameters are involved in the choice of a specimen for sporting activity, the most important one is

the absence of any disorder or malformation of the limbs (Love et al. 2006; Sánchez, Gómez, Peña, et al. 2013). Morphological defects in any species, including those of the horse, have origins which are both polygenic (Lampe et al. 2009; Distl 2013) and multifactorial (Árnason and Dale Van Vleck 2000). Currently, the characterisation of the equine genome and the identification of genes mutations associated with musculo-skeletal disorders, together with classical selection, has allowed important advances in the genetic improvement of these types of hereditary diseases (Farries et al. 2019).

A total of 43,358 PRE horses were evaluated, of which between 40,607 in knock-kneed defect and 13,286 in divergent defect (Table 1), while a total of 6331 horses (14.60%) did not have any hock defect and 23,631 horses (54.50%) did not have any knee defect. The prevalence of animals affected with hock defects ranged from 20.98% (divergent) to 74.12% (convergent). The prevalence of animals affected with knee defects ranged from 3.31% (bench-knee) to 26.47% (knock-knee).

In the PRE horse, hock defects are more prevalent than knee defects, with the most prevalent being the convergent hock defect. This defect can lead to a narrow rump, accompanied by weak muscles, and can result in the horses having little resistance to constant, high-intensity exercises (Stashak 2002). The hocks can even hit each other if the defect is very pronounced, which hinders the horse's movement considerably. On the other hand, horses with divergent hocks experience greater pressure on the external part of the joint, causing muscular pain.

The gait is irregular, and the power of impulsion can be considerably reduced. Likewise, a horse that presents the defect of convergent or divergent hocks has greater difficulty in achieving correct gaits (Sánchez-Guerrero et al. 2017). The most prevalent knee defect in the PRE horses is the knock defect, which results in an unstable, slower gait since this

defect decreases the base of support and consequently decreases balance. This deformation causes the force and weight to rest on the internal part of the carpus, causing constant overexertion in the muscles (anterior extensor and oblique of the metacarpus) (Auer and von Rechenberg 2006). The prognosis is good if the deviation is mild and treated at an early age, but it can affect the horse's performance due to the abnormal stress placed on the tendons and muscles (Stashak 2002). On the other hand, horses with bench defect have poor endurance and suffer from fatigue when performing constant muscular effort, with less balanced gaits. The prevalence of hock defects and knee defects in PRE was greater than the prevalence of conformational limb defects obtained by Holmström et al. (1990) in Swedish Warmbloods (5.6%), and similar to that obtained by Robert et al. (2013) in French Trotters (47%) and by Mostafa et al. (2019) in Jumping Thoroughbreds (58%). The PRE horse has traditionally been used for leisure and field work, with very little participation in sporting events. Therefore, there had been no selection pressure to eliminate these types of defects. It was in the year 2012 when these defects were introduced in the official performance control of the breed to be included in the genetic evaluations in order to reduce the prevalence in the population.

Bone morphology and conformation affect welfare, utility and functional ability in horses (Mawdsley et al. 1996), which in turn affect the animal's survival, and are related to movement dynamics (Dellanini et al. 2003; Van Weere and Crevier-Denoix 2006). Several authors have shown that between 50% and 70% of horse deaths are due to skeletal defects (Wallin et al. 2000; Perkins et al. 2005; Egenvall et al. 2006; Dyson et al. 2008; Murray et al. 2010; Sloet van Oldruitenborgh-Oosterbaan et al. 2010), and there are many predisposing factors. Therefore, good conformation without morpho-skeletal defects is the most

Table 1. Number and proportions of Pura Raza Española horses according to the class of conformational limb defects and the significance level for the difference within unaffected (class 0) vs affected animals (class 1 + 2).

		Number of horses (%)					
Limbs conformational defects		Total	Class 0	Class 1	Class 2	Class 1 + 2	<i>p</i> -value
Hock defects	Closed	31,040	24,184 (77.91%) ^b	4786 (15.42%)	2070 (6.67%)	6856 (22.09%) ^a	<.0001
	Open	36,502	24,184 (66.25%) ^b	9109 (24.95%)	3209 (8.79%)	12,318 (33.75%) ^a	<.0001
	Convergent	40,570	10,498 (25.88%) ^b	21,379 (52.70%)	8693 (21.43%)	30,072 (74.12%) ^a	<.0001
	Divergent	13,286	10,498 (79.02%) ^b	1956 (14.72%)	832 (6.26%)	2788 (20.98%) ^a	<.0001
Knee defects	Buck	32,556	30,431 (93.47%) ^b	1699 (5.22%)	426 (1.31%)	2125 (6.53%) ^a	<.0001
	Calf	39,503	30,431 (77.03%) ^b	7190 (18.20%)	1882 (4.76%)	9072 (22.97%) ^a	<.0001
	Bench	30,879	29,858 (96.69%) ^b	820 (2.66%)	201 (0.65%)	1021 (3.31%) ^a	<.0001
	Knock	40,607	29,858 (73.53%) ^b	8323 (20.50%)	2426 (5.97%)	10,749 (26.47%) ^a	<.0001

Class 0 = absence of the defect; class 1 = slight presence of the defect; class 2 = the most pronounced degree of defect; class 1 + 2 = affected animals.
^{a,b}Different superscript letters indicate significant difference between groups ($p < .05$).

important trait when marketing a horse (Hennessy et al. 2008).

The most significant factors in the eight conformational defects can be seen in Table 2, using a generalised non-linear model. The age factor has been introduced in the genetic model, since aplomb deformities which are acquired tend to worsen with age (Blunden et al. 2006) or, in contrast, if they are congenital diseases, may appear at an early age (Smith 2010; Robert et al. 2013). Coat colour has also been included; different authors have described how certain coats present a higher incidence of morphological defects, such as cresty neck (Sánchez et al. 2017), vitiligo, melanoma (Sánchez-Guerrero et al. 2017; Sánchez-Guerrero, Solé, et al. 2019) and ewe neck (Ripolles et al. 2020). Depending on the coat colour, horses have different origins and founders, and it is also related to the conformation of the horse, for that reason it is recommended that this parameter be included in genetic evaluation models (Poyato-Bonilla et al. 2018; Junqueira et al. 2021). Furthermore, it has been ascertained that there is considerable morphological variation between PRE horses of different coat colours through a methodology that combines molecular markers and expert phenotypic characterisation (Sánchez-Guerrero, Negro, et al. 2019).

Other factors analysed were the birth stud size of the herd and the geographic area. The development of conformation defects depends, to a certain extent, on the type of management, feeding and training of the horses. Many authors have evidenced the importance of feeding and management on morphological development in horses (Lepeule et al. 2009; McIntosh et al. 2018; Valberg 2020) and have found a correlation between a rapid growth rate, associated to an intensive rearing system, and the occurrence of deformities in the aplomb and skeletal developmental abnormalities (Dik et al. 2010; Mendoza et al. 2016). The proportionality index depends on the musculoskeletal development of the horse, and a high or low proportionality index can therefore condition the

appearance of defects in the joints, and, in turn, in hock and knee defects.

The number of observations per each level of the factors are shown in Table 3. As can be seen, all the effects studied were significant for some of the defects analysed (age, gender, coat colour, birth stud size, geographical area, inbreeding, proportionality index), with significance coefficients below 0.05. In general, the class of effects that presents the highest proportion of limb conformation defects, are: females, this could be due to the fact that the selection patterns by breeders are stricter in stallions, since they are the protagonists in most of the competitions; young horses, because breeders have discarded adult animals with defects; chestnut coat, over time there has been an increasing preference for dark coat colour horses, which has led to less thorough selection of defects by breeders in this coat colour (Poyato-Bonilla et al. 2018); stud in Spain with a big birth stud size, small stud farms are dedicated to equine competitions, therefore, they usually have horses without conformation defects and with the best possible morphological conditions; inbreeding index higher than 12.50%, high inbreeding can favour the appearance of limb conformation defects, possibly due to the potential for inbreeding depression and the appearance of homozygous recessive genes that had not been expressed until now (Poyato-Bonilla et al. 2020); proportionality index lower than 98, being the animals that move away from the most proportionate animal. However, many horses presenting a high level of hock and knee defects have not been analysed, as they have either been slaughtered or have not survived; therefore, the prevalence of the defect could in fact be much higher than the figure found in this study.

These conformation defects predispose horses to serious musculoskeletal problems (Globe 1992), which have been studied from a genetic point of view in other equine breeds (Maremmano by Pieramati et al. 2003; Hanoverian by Stock et al. 2005 and Hilla and Distl 2014; Thoroughbreds by Love et al. 2006 and Welsh et al. 2013; Swiss Warmblood by Lauper et al.

Table 2. Generalised Non-Linear Model (GLZ) between conformation limb defects and effects, using a multinomial logic test (Wald Statistic).

	Defects	Age	Gender	Coat Colour	Birth stud size	Geographical area	Inbreeding Coefficient	Proportionality Index
Hock defects	Closed	0.0510	0.0300	0.0020	<0.0001	<0.0001	<0.0001	<.0001
	Open	0.0150	<0.0001	<0.0001	0.2040	<0.0001	0.0190	<.0001
	Convergent	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.7800	<.0001
	Divergent	<0.0001	0.0130	0.0090	0.1940	<0.0001	0.7920	<.0001
Knee defect	Buck	0.6300	<0.0001	0.1780	<0.0001	<0.0001	<0.0001	<.0001
	Calf	<0.0001	<0.0001	<0.0001	0.0600	<0.0001	0.1250	<.0001
	Bench	0.0710	<0.0001	0.0167	0.0185	<0.0001	0.5280	<.0001
	Knock	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.6320	<.0001

Table 3. Total number of observations and percentage of Pura Raza Española horses affected by conformation limb defects (horses in classes 1 and 2), according to the effects, after an association frequency analysis of all the effects (Maximum-Likelihood Chi-square).

Effects	N	Population affected hock defects (%)				Population affected knee defects (%)				
		Closed	Open	Conv.	Diver	Buck	Calf	Bench	Knock	
Age	Young (<4)	22,403	16.14 ^a	28.50 ^b	70.09 ^b	7.26 ^b	5.07 ^a	22.61 ^b	2.43 ^a	27.10 ^b
	Adult (≥4)	20,955	15.46 ^a	28.32 ^a	68.58 ^a	5.54 ^a	5.14 ^a	20.93 ^a	2.48 ^a	24.46 ^a
Gender	Stallions	14,331	16.14 ^b	26.91 ^a	67.46 ^a	6.63 ^b	6.81 ^b	20.01 ^a	2.07 ^a	28.17 ^b
	Female	29,027	15.65 ^a	29.15 ^b	70.30 ^b	6.33 ^a	4.27 ^a	22.67 ^b	2.64 ^b	24.67 ^a
Coat Colour	Bay	15,963	15.72 ^b	29.29 ^b	70.02 ^b	6.35 ^a	5.26 ^a	21.27 ^a	2.37 ^{a,b}	25.75 ^{b,c}
BSS	Chestnut	2863	13.13 ^a	34.06 ^c	70.63 ^{a,b}	6.39 ^a	5.41 ^a	19.87 ^a	1.90 ^a	23.77 ^{a,b}
	Black	5404	14.17 ^a	29.15 ^b	67.02 ^a	7.85 ^b	5.64 ^a	21.40 ^{a,b}	2.75 ^b	23.55 ^a
	Grey	19,128	16.75 ^c	26.63 ^a	69.28 ^{a,b}	6.10 ^{a,b}	4.77 ^a	22.64 ^b	2.52 ^b	26.85 ^c
G.Area	<3	7177	13.21 ^a	29.34 ^a	68.78 ^a	5.71 ^a	4.45 ^a	21.98 ^a	2.79 ^b	24.70 ^a
	3-9	21,863	15.63 ^b	28.68 ^a	68.33 ^a	6.48 ^a	5.05 ^{ab}	22.15 ^a	2.56 ^b	26.42 ^b
	>9	14,318	17.39 ^c	27.52 ^a	71.22 ^b	6.72 ^a	5.51 ^b	21.16 ^a	2.12 ^a	25.47 ^{a,b}
F (%)	Spain	28,151	15.82 ^c	29.33 ^b	71.94 ^c	4.76 ^a	5.34 ^{bc}	21.29 ^a	2.13 ^a	25.97 ^b
	Europe	4138	11.26 ^a	31.56 ^b	67.79 ^{bc}	5.39 ^b	5.85 ^c	20.54 ^a	2.45 ^a	23.85 ^a
	S.A.+ C.A.	2809	14.03 ^b	29.19 ^b	57.42 ^a	12.46 ^c	4.58 ^{ab}	25.12 ^c	3.61 ^b	23.47 ^a
P.I.	USA + Mex	826	18.68 ^d	23.41 ^a	65.41 ^b	10.59 ^b	4.17 ^a	22.90 ^b	3.10 ^b	27.12 ^b
	<3.125	977	11.98 ^a	30.71 ^b	68.58 ^a	5.02 ^a	7.51 ^b	21.14 ^a	2.90 ^a	24.89 ^a
	≥3.125 to < 6.25	23,517	15.28 ^b	28.43 ^c	69.47 ^a	6.48 ^a	5.00 ^a	21.75 ^a	2.44 ^a	25.37 ^a
	≥6.25 to < 12.50	13,988	16.14 ^c	28.75 ^{b,c}	69.32 ^a	6.48 ^a	4.92 ^a	21.69 ^a	2.51 ^a	26.03 ^a
P.I.	≥12.50	4876	18.21 ^d	26.87 ^a	69.09 ^a	6.36 ^a	5.64 ^a	22.43 ^a	2.27 ^a	27.54 ^a
	<98	5905	21.64 ^c	27.57 ^a	68.45 ^a	5.61 ^{ab}	3.48 ^a	29.06 ^b	3.08 ^b	32.02 ^c
	98-100	20,721	15.44 ^b	29.64 ^b	70.68 ^b	6.61 ^a	4.62 ^b	21.04 ^a	2.25 ^a	22.53 ^b
>100	16,732	14.22 ^a	27.18 ^a	68.04 ^a	6.50 ^b	6.29 ^c	20.12 ^a	2.48 ^a	27.72 ^a	

N: Number of observations; Conv.: Convergent hock; Diver.: Divergent hock; BSS: Birth stud size; G.Area: Geographical area; S.A.: South America; C.A.:Central America; Mex:México; F: Inbreeding coefficient; P.I.: Proportionality index.
^{a,b,c}Different superscript letters indicate significant difference between groups ($p < .05$).

Table 4. Heritability coefficient (h^2), genetic correlations (r_g) and their standard deviations (s.d.) for hock defects and knee defects in Pura Raza Española horses.

	Approach	Defects	h^2 (s.d.)	Defects	r_g (s.d.)
Hock defects	1	Closed	0.40 (0.017)	Closed vs Convergent	0.51 (0.038)
		Open	0.27 (0.015)	Closed vs Divergent	0.38 (0.137)
		Convergent	0.26 (0.010)	Open vs Convergent	0.29 (0.037)
		Divergent	0.42 (0.025)	Open vs Divergent	0.28 (0.070)
		Closed/Open hock	0.21 (0.060)		
Knee defects	1	Convergent/Divergent hock	0.24 (0.031)		
		Buck	0.33 (0.024)	Buck vs Bench	0.42 (0.203)
Knee defects	2	Calf	0.32 (0.014)	Buck vs Knock	0.13 (0.061)
		Bench	0.25 (0.036)	Calf vs Bench	0.20 (0.133)
		Knock	0.38 (0.013)	Calf vs Knock	0.70 (0.276)
		Buck/Calf kneed	0.14 (0.057)		
		Bench/Knock kneed	0.18 (0.069)		

2017 and Arabian by Comparini et al. 2019). In this context, the estimation of genetic parameters is essential in order to manage the breeding program efficiently. Good knowledge of genetic parameters is directly linked to its success, as they condition the type of selection that can be carried out (Sánchez, Gómez, Molina, et al. 2013). Genetic parameters of hock and knee defects can be seen in Table 4.

Heritability estimates were moderate for limb conformation defects, ranging from 0.14 to 0.42 (left part of Table 4). In Approach_1, heritability for hock defects ranged between 0.26 (convergent) and 0.42 (divergent) and for knee defects between 0.25 (bench) and 0.38 (knock). In Approach_2, heritability for hock defects ranged between 0.21 (Closed/Open: Hock in

lateral view) and 0.24 (Convergent/Divergent: Hock in rear view) and for knee defects between 0.14 (Buck/Calf: knee in lateral view) and 0.18 (Bench/Knock: knee in frontal view). The heritability obtained will allow the prevalence of these defects to be reduced by genetic selection. Using the hock and knee defects as a selection criterion in the PRE's breeding program could contribute to the eradication of major conformation defects which make the animals unsuitable for sport and cause a significant economic loss to the breeding herds.

The heritabilities obtained using Approach_1 are higher than those obtained using Approach_2. The works found in the literature which have studied the estimation of genetic parameters of the

conformational limb defects have used a scale similar to that used in our Approach_2, where the opposing effects occupy the extremes of the scale, and the central class corresponds to 'absence of defect'. In our work, in general, the heritabilities of hock and knee defects were similar to those obtained by Rustin et al. (2009) in Belgian warmblood horses (0.22–0.35); slightly lower to the heritabilities of limbs defects obtained by Dolvik and Klemetsdal (1999) in Norwegian Trotters (0.27–0.65), by Love et al. (2006) in Thoroughbreds (0.26–0.99) and slightly higher than those in PRE (0.08–0.19) obtained by Sánchez, Gómez, Molina, et al. (2013), in Italian Heavy Draught (0.03–0.12) obtained by Folla et al. (2020) and in Pura Raza Menorquina (0.09–0.20) obtained by Perdomo-González et al. (2022).

If we compare the heritabilities obtained in this work for the heritabilities of the conformational defects in the PRE, it can be seen that they are in a similar range. In particular, a heritability of 0.37 was found for cresty neck (Sánchez et al. 2017), while the heritability for ewe neck ranged from 0.23 to 0.34 (Ripolles et al. 2020).

Genetic correlations within defects (right part of Table 4) were positive in the moderate-high range in PRE, ranging between 0.28 (open vs divergent) and 0.51 (closed vs convergent) for hock defects, and between 0.13 (buck vs knock) and 0.70 (calf vs knock) for knee defects. There is a close relation between limb conformation and predisposition to lameness (Kane et al. 2000; Stashak 2002; Shearer et al. 2004; Ross and Dyson 2010). On the other hand, as can be seen in Table 5, the presence of hock defects may favour the presence of knee defects and vice-versa. In Table 6, the highest positive genetic correlations were between closed hock/knock-knee (0.59) and open hock/buck-knee (0.41). These defects often occur in parallel in the same animals and often present problems of severe mobility and lameness (Stashak 2002). The highest negative genetic correlations were between closed hock/bench-knee (−0.25) and open hock/calf-knee (−0.18).

Table 5. Genetic correlations and their standard deviations (in parenthesis) between hock defects and knee defects of Pura Raza Española horses.

	Hock defects			
	Closed	Open	Convergent	Divergent
Knee defects				
Buck	0.10 (0.079)	0.41 (0.055)	−0.06 (0.051)	0.39 (0.094)
Calf	0.39 (0.043)	−0.18 (0.049)	0.15 (0.034)	0.23 (0.080)
Bench	−0.25 (0.160)	0.25 (0.123)	0.12 (0.106)	0.07 (0.025)
Knock	0.59 (0.033)	−0.14 (0.042)	0.21 (0.029)	0.20 (0.069)

There are many works that use the methodology of Linear Morphological Qualification in equine breeds as a standard method for morphological performance control (Rustin et al. 2009; Sánchez, Gómez, Molina, et al. 2013; Novotná et al. 2017; De Oliveira et al. 2018; Ghezelsoufou et al. 2018; Viklund and Eriksson 2018; Ablondi et al. 2020; Folla et al. 2020). All the works found use a linear scale, with more or less classes, ranging from closed to open in lateral view hock defect, convergent to divergent in rear view hock defect, buck to calf in lateral view knee defect and bench to knock in frontal view knee defect. In these works, moderate positive genetic correlations have been found between convergent/divergent hock vs buck/calf knee (0.10–0.39) and between open/closed hock vs bench/knock knee (0.10–0.56). Moreover, genetic correlations, ranging from −0.06 to −0.40, have been found between convergent/divergent hock vs bench/knock knee in Belgium Warmblood (Rustin et al. (2009), in Italian Heavy Draught (Folla et al. 2020) and Pura Raza Menorquina (Perdomo-González et al. 2022). In the work of Sánchez, Gómez, Molina, et al. (2013) in PRE horses, the correlations found were low, possibly due to the impossibility of finding logical correlations between these types of variables that use a scale with opposing defects at their extremes.

Finally, in order to assess the differences between the estimations of breeding values for conformational limb defects according to the approach used (Approach_1: scale from 0 to 2 vs Approach_2: scale from −2 to 2). The proportion of coincident animals was determined for the top 5% and 20% of the estimated breeding values (EBV), taking into account just those animals with reliability equal or greater than 0.20 and 0.60 (Table 6).

As can be seen, open-hock defect and the knock-knee defect had the highest percentage of coincident

Table 6. Percentage of coincident animals within the upper 5 and 20 percentile of the estimated breeding values (EBV), with a reliability greater or equal than 0.20 and 0.60, between the genetic models used in Approaches 1 and 2.

	Reliability \geq 0,20		Reliability \geq 0,60	
	EBV 5%	EBV 20%	EBV 5%	EBV 20%
Hock defects				
Closed	67.35	69.12	62.50	57.14
Open	95.89	93.69	75.00	77.77
Convergent	75.25	78.74	66.66	70.10
Divergent	69.02	71.02	25.00	35.00
Knee defects				
Buck	32.86	45.59	33.33	50.00
Calf	37.59	46.63	6.60	20.39
Bench	27.75	31.89	33.33	50.00
Knock	94.12	91.17	92.85	96.43

Approach_1: scale from 0 to 2; approach_2: scale from −2 to 2.

animals according to the approach used. Conversely, the lowest percentage of coincidence animals between the two models was for the divergent hock defect and the calf-knee defect. The fact that the coincidence of animals with a higher EBV for each of the defects is low, according to the methodology used for the estimation, makes it evident that the use of the linear scale (e.g. Approach_2, in which opposing defects are placed at the extremes, for example, open and closed hock), is not suitable for estimating the EBV of a given defect. This could be because, although there may be a group of common genes that determine the occurrence of conformational limb defects, there may also be specific genes that determine the presence of specific defects.

Conclusions

In the PRE horse, hock and knee defects have a high prevalence because, until very recently, they were not included in the official performance monitoring of the Breed Improvement Program, finding that hock defects are more prevalent than knee defects. The most prevalent hock defect is the convergent defect, while the most prevalent knee defect is the knock defect. Risk factors (age, gender, coat colour, geographical area, size breeder's stud farm, inbreeding and proportionality index) are also involved in the development of conformational limb defects, being more prevalent in horses which are young, female, with a bay coat, raised in Spain, with a certain birth stud size (more than 9 foals per years), with an inbreeding value of over 12.50% or with a proportionality index less than 98.

The genetic base of conformational limb defects in PRE, which presents moderate heritability, shows that the prevalence of this defect could be reduced by genetic selection using the breeding values in all PRE horses. We recommend evaluating each of the defects independently (as in Approach_1) and not using the linear scale where the opposite defects are located at the extremes (Approach_2), which has been used systematically in many horse breeds.

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Ethical approval


The approval of the work by an ethics committee was not required.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, MRL, upon reasonable request.

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