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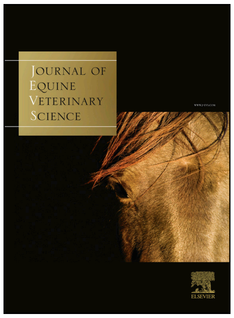

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PII: S0737-0806(18)30135-7

DOI: 10.1016/j.jevs.2018.07.009

Reference: YJEVS 2566

To appear in: *Journal of Equine Veterinary Science*

Received Date: 21 March 2018

Revised Date: 12 July 2018

Accepted Date: 14 July 2018

Please cite this article as: António da Silva Faria R, Andrade Vicente AP, Duarte Guedes dos Santos RI, Maiorano AM, Curi RA, Loyola Chardulo LA, Vasconcelos Silva JA II, Genetic diversity of Lusitano horse in Brazil using pedigree information, *Journal of Equine Veterinary Science* (2018), doi: 10.1016/j.jevs.2018.07.009.

Genetic diversity of Lusitano horse in Brazil using pedigree information

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Abstract – This study aimed to evaluate population parameters and to describe the genetic diversity of the Lusitano breed in Brazil using pedigree data. Two populations evaluated: total population (TP) containing 18,922 animals, and reference population (RP) composed of a part of TP containing 8,329 animals, representing the last generation. The generation interval (10.1 ± 5.1 years) was in the range for horse populations. Pedigree completeness in RP shows almost 100% filling in the three most recent generations, indicating improvement in the pedigree data and accuracy of the results. The inbreeding coefficient (4.46%) and average relatedness (5.97%) for RP, indicating control on the part of breeders. The effective population size was 89 (TP) and 90 (RP). The effective number of founders (fe) of 33 and 29, effective number of ancestors (fa) of 30 and 26, and effective number of founder genomes (fg) of 19 and 15 for TP and RP, respectively, indicating a reduction of genetic variability in the last generations. The total number of ancestors that explains 100% of the genetic diversity in the Lusitano breed in Brazil was 427 (TP) and 341 (RP). The reproductive parameters, probabilities of gene origin showing loss of variability in the last generations and the genetic contributions of ancestors suggest the need to monitor genetic diversity over time in breeding programs in order to allow control of the next generations and to increase their variability.

Keywords: ancestors; demographic characterization; effective population size; founders; inbreeding.

1. Introduction

The Puro Sangue Lusitano (PSL), also known as the Lusitano horse, represents a breed with a rich and ancient history, originated in southern Portugal on the Iberian Peninsula. Its evolution is essentially due to their use for working cattle and cutting bulls [1]. Although records date back to 1824, the studbook of the Lusitano breed was officially established in 1967 [2], a time when the main Iberian breeds, Portuguese and Spanish, were separated and received the denomination PSL in Portugal and Pura Raza Española (PRE) in Spain.

The main studbook of the Lusitano breed is managed in Portugal by the Portuguese Lusitano Horse Breeder Association (APSL in the Portuguese acronym), which has the largest number of birth records of the breed in the world [2]. Brazil is the country with the second largest number of animal records of this breed. This statistic was determined after the reciprocity partnership between APSL and the Brazilian Lusitano Horse Breeder Association (ABPSL in the Portuguese acronym) was established in 1991. This agreement permitted all Lusitano horses registered in the Brazilian studbook to be equally registered in the Portuguese studbook, which contains the birth records of all countries.

Studies investigating population parameters of the Lusitano breed have been conducted only with data from the APSL studbook (Portugal) for all Lusitano horses registered in the world [2,3]. Thus, data on the population statistics and genetic diversity of the Lusitano breed in Brazil are lacking.

Parameters such as pedigree completeness [4], generation interval, inbreeding coefficient [5], and probability of gene origin [6] are important to design strategies for the selection and improvement of animals. The data generated permit to verify genetic diversity and its changes over time [7]. The complete or partial results of genetic diversity and population parameters are reported in the literature for various horse breeds, including Andalusian [8] , Holstein [9], Paint-Horse [10], Hanoverian [11], Spanish Arab Horse [12], Lipizzaner [13] and Old Kladruber [14]. In Brazil, studies were conducted on the Pantaneiro [15], Mangalarga [16] Campolina [17], Brazilian Sport Horse [18] and Quarter Horse [19] breeds.

This study aimed to evaluate the population parameters of the Lusitano horse in Brazil in order to observe the genetic diversity and to contribute to the knowledge and development of this breed in Brazil and to compare our results with the ones obtained by different authors for the worldwide population [2,3], providing the information necessary to implement a breeding program of the breed in Brazil.

2. Material and methods

2.1. Data and computer programs

The pedigree file of the Lusitano breed containing information about the animal's name, sire and dam, sex, date of birth and origin was provided by ABPSL. Animals born and registered in Brazil between 1912 and 2012 were considered and made up the total population (TP) of 18,922 animals (48.1% males, stallions and geldings) and the reference population (RP) consisting of 8,329 animals (51.0% males, stallions and geldings), corresponding to the birth records from 2003 to 2012. RP was used as the reference of the active population, representing the last generation, which was equal to one mean generation interval (10.1 years) and contained animals (stallions and mares) that potentially could transmit their genes to the next generation.

Data preparation and statistical analysis were performed with the MEAN and FREQ procedures of the SAS program [20]. The population and reproductive parameters, probability of gene origin and genetic diversity were obtained with the ENDOG V4.8 program [21].

2.2. Reproductive parameters and Generation interval

The existence of imported animals registered in the studbook of ABPSL permitted to observe their use as breeding stock and to compare it with that of sires and dams born in Brazil. The coefficients of variation for the mean number of offspring, age at first and last progeny and time in reproduction obtained for stallions and mares explain the high standard deviation between the different parameters analyzed. These parameters were not calculated for RP because of the small number of imported breeding animals during this period and because the population is still in reproduction.

The generation interval (GI) was obtained based on the mean age of the parents at the birth of offspring that reproduced [22], and was calculated for the four different paths of selection: father-son, father-daughter, mother-son and mother-daughter and total parent-offspring.

2.3. Quality of pedigree data

The quality of the information in the ABPSL studbook is reported in two ways: 1) pedigree completeness summarizes the proportion of known ancestors in each ascending generation per descendant and was calculated as proposed by MacCluer et al [4], in which ancestors without progenitors in the pedigree were considered founders [21]; 2) based on the mean number of generation equivalents (GE), computed over the sum of all known ancestors by calculating $(½)^n$, where n is the number of generations between the animal and each known ancestor [7].

2.4. Parameters related to the inbreeding coefficient

The inbreeding coefficient (F) defines the probability that an individual has two identical alleles by descent and was calculated using the algorithm proposed by Meuwissen and Luo [23].

The average relatedness (AR) coefficient of each animal was described by Gutiérrez and Goyache [21] as the representation of a given animal in the pedigree and was obtained based on the probability that a randomly selected allele of the population belongs to a given animal.

The increase in inbreeding (ΔF) for each generation was obtained as follows:

$\Delta \mathrm{F} = (\mathrm{F_t} - F_{t-1})/(1 - F_{t-1})$ (1) where F_t and F_{t-1} are the average inbreeding in generations t and $t-1$, respectively.

According to Falconer and Mackay [24], the effective size of a population (N_e) is defined as the number of individuals of a population with a non-ideal structure that would give origin to a certain rate of consanguinity if its structure were ideal (e.g., equal number of males and females, absence of selection, random matings, etc.). Using ΔF, N_e was estimated considering $N_e = 1/2\Delta F$, which represents the number of animals that equally contribute to the next generation and that would promote a similar increase in inbreeding in the population studied [21]. In addition, N_e was also calculated based on the individual increase in inbreeding as suggested by Gutiérrez et al [25] and was used for the calculation of genetic drift.

2.5. Probability of gene origin and genetic drift

The effective number of founders (f_e) is obtained by measuring the contributions of the most influential founders. Lacy [26] defines f_e as the expected number of founders that contribute equally and produce the same genetic diversity in the population studied. This parameter was calculated using the formula

$f_e = 1/\sum_{k=1}^{f} q_k^2$ (2) where q_k is the probability of gene origin of founder k.

The effective number of ancestors (f_a) represents the minimum number of ancestors (founders or not) necessary to explain the full genetic diversity of the population [6]. This parameter was calculated as

$f_a = 1/\sum_{j=1}^{f} p_j^2$ (3) where p_j is the marginal contribution of ancestor *j*. The marginal contribution is the additional genetic contribution made by an ancestor that was not explained by another previously chosen ancestor [6].

The effective number of founder genomes (f_g) is defined as the number of sires and dams that contribute equally to the population structure and produce identical genetic diversity without the loss of alleles [27]. This parameter was estimated as proposed by Caballero and Toro [28] using the formula

$f_g = 1/2C$ (4) where *C* is the average coancestry between individuals of the population.

Genetic drift is the random change in allele frequencies in a population, which occurs at a higher intensity when the population undergoes a drastic reduction in its effective size [24]. According to Sørensen et al [29], a ratio of $f_e/N_e > 0.5$ in a population indicates the occurrence of changes in genetic drift. One approach to evaluate genetic drift is the observation of bottleneck effects. The f_e/f_a ratio should be close to 1 if important bottlenecks have not occurred in the population [6]. Stabilization of genetic drift in a population can be observed when f_e is close to $N_e/2$, suggesting a greater representation of founders [28].

2.6. Genetic conservation index

The number of founders represented in the contributions received by an individual and the balance between them were evaluated by computing the genetic conservation index (GCI) of all animals [30]. It is supposed that higher indices correspond to animals with a more balanced representation from a large number of founders, i.e., greater genetic conservation of the breed. The GCI was computed for each animal using the formula

$GCI = 1/\sum P_i^2$ (5) where P_i^2 is the proportion of genes of founder animal *i* in the pedigree [21]. The mean of the populations was then calculated.

3. Results

3.1. Data analysis and distribution of birth records

In TP, 87.3% of the animals were born in Brazil after 1967 and 4.0% were imported. The remaining animals are ancestors of both origins, which are registered only as parents in the database of ABPSL. In RP, 99.6% of the animals studied were born in Brazil and the remaining 0.4% are derived from imported animals and ancestors.

The first birth registered by ABPSL and recognized by APSL was the female Azambuja in 1967 and the first male was Zapata in 1969, both half-sibs of the mare Zaza. Between 1967 and 1985, the births of only 305 animals were recorded (Figure 1). A constant increase was observed after 1986 and the number of registered births per year reached three digits for the first time. Stabilization of the growth trend occurred in 2002, with reduced oscillations (approximately 5%) in the following eight years. The largest number of registered births was observed in 2006 (966 animals). A marked decline in the number of births was seen in the last three years (2010 to 2012) of the study (Figure 1).

Births were observed in all months but there was wide oscillation. In TP, only 4.6% of births occurred in the ideal month (July). The months with the largest number of births were October (16.5%), September (15.7%), and November (15.3%). The first, second and last quarter of the year concentrated 17%, 5% and 45% of births, respectively.

3.2. Reproductive parameters and Generation interval

In the database, there were 16,511 (50.4% males, stallions and geldings) Lusitano horses born in Brazil (national) and 781 (32.9% males, stallions and geldings) imported animals (e.g., Portugal and Mexico). Of the 8,319 national males, only 10.1% had offspring, while 257 (72.2%) of the imported males were used as stallions. Of the 8,192 national females, 37.7% had offspring and 96.2% of the 524 imported females were used as mares.

All animals born in Brazil (16,511) were sired by 1,115 stallions; 57.5% of the offspring had a sire of Brazilian origin, 40.8% had an imported sire, 1.6% were obtained by artificial insemination, and the sire was unknown in 0.1%. Among the 3,594 breeding mares that contributed to the Brazilian population, 77.2% of the offspring had a dam of Brazilian origin, 22.6% had an imported dam, 0.1% were obtained by embryo transfer, and the dam was unknown in the remaining 0.1%.

There was a greater utilization of females, with a ratio of 3.1 mares (4,644) per stallion (1,507). This ratio was 4.5 mares per stallion (210 stallions and 946 mares) in the most productive year (2006). The mean number of offspring (Table 1) considering only breeding animals was 12.3 and 4.0 for stallions and mares, respectively. Considerable differences were observed in the maximum number of offspring, with stallion Afiançado de Flandes having 419 offspring registered, the largest number for the whole breed worldwide.

The mean age of stallions at the birth of their first progeny was 7.0 years and mares had their first foal at 5.9 years (Table 1). The mean age at the birth of the last progeny was similar in stallions and mares, with a difference of 0.2 years (Table 1). The difference between the birth of the first and last offspring, indicating the time in reproduction, was 3.7 ± 5.0 years for stallions and 4.6 ± 5.1 years for mares.

High standard deviations were observed for all reproductive parameters analyzed (Table 1). A standard deviation higher than the mean was found for the number of offspring per stallion, which was almost the double of the mean value. The coefficients of variations indicated a high level of dispersion, which were 199.1% (number of offspring), 54.3% (age at first progeny), 53.3% (age at last progeny) and 135.1% (time in reproduction) for stallions, and 85.0% (number of offspring), 52.5% (age at first progeny), 50.5% (age at last progeny) and 110.9% (time in reproduction) for mares.

The overall mean GI was 10.1 ± 5.1 and 10.2 ± 5.0 years for TP and RP, respectively (Table 2). When the four paths of selection were considered, shorter GIs were observed for the mother-offspring paths in TP. The same trend was not found in RP, with longer and shorter GIs between fathers and their offspring. The standard deviations were high (Table 2) and an approximate dispersion of the results was observed in both populations studied.

3.3. Quality of pedigree data

The quality of the information of the ABPSL studbook based on average pedigree completeness (Figure 2) was close to zero from the 15th (oldest) to the 11th generation in both populations and in the three most recent generations (1st, 2nd and 3rd), with pedigree completeness of 97.7%, 95.4% and 92.2% in TP, respectively, and of 99.8%, 99.5% and 98.2% in RP. The number of GE was 5.7 ± 1.4 and 6.4 ± 0.7 for TP and RP, respectively.

3.4. Parameters related to the inbreeding coefficient

There were 14 matings between full-sibs, 607 between half-sibs, and 149 between parents and offspring. The F value was 4.06% ± 4.94% in TP and a slight increase of 0.40% to 4.46% ± 4.34% was observed in RP. The AR coefficient was 5.41% ± 1.69% in TP and increased by 0.56% to 5.97% ± 1.20% in RP. The ΔF per generation was 0.96% ± 2.09% in TP, while a lower value (0.85% ± 0.90%) was observed in RP (Table 3). The percentage of animals with F different from zero was 88.7% in TP and 98.0% in RP (Table 3). Only two non-inbred animals were born in the last year evaluated (2012).

The N_e obtained based on ΔF is the most common parameter in the literature and was 89 and 90 in TP and RP, respectively. Using the individual increase in inbreeding, N_e values of 52 (TP) and 59 (RP) were obtained.

3.5. Probability of gene origin and genetic drift

The genetic variability of the populations demonstrated by the probability of gene origin (Table 3) indicated an effective number of founders (f_e) of 33, ancestors (f_a) of 30, and founder genomes (f_g) of 19 in TP. These values decreased to 29, 26 and 15, respectively, in RP.

Analysis of possible genetic drift in the populations showed an f_e/N_e ratio of 0.64 in TP and of 0.49 in RP (Table 3). When the absence of bottleneck effects was evaluated, f_e/f_a ratios of 1.10 (TP) and 1.12 (RP) were obtained. The f_e ~ (N_e/2) values were 33 ~ 26 and 29 ~ 30 for TP and RP, respectively.

3.6. Genetic conservation index

The GCI indicated a mean number of founders per individual of 9.6 ± 2.9 and 9.7 ± 2.9 in TP and RP, respectively, with a maximum GCI of 22.1 founders. In RP, GCI values less than 7.7 and higher than 11.7 corresponded to the 25th and 75th percentiles, respectively.

3.7. Genetic contributions

The total number of founders (Table 3) of the Lusitano breed in Brazil represents 2.7% (TP) and 5.4% (RP) of each population and the total number of ancestors 2.3% (TP) and 4.1% (RP). The cumulative genetic contribution (Table 3) of the 10, 50 and 100 most influential founders exhibited a reduced increase in RP by 0.8%, 3.9% and 3.0%, respectively, compared to TP. The cumulative marginal genetic contributions of ancestors were also slightly increased in RP by 5.4%, 2.6% and 1.4% for the 10, 50 and 100 major ancestors (Table 3).

Figure 3 shows the genetic contributions of the 10 most influential founders of the Lusitano Breed in Brazil since the first birth registered (1967) until 2012, which are Agareno, Destinado IV, Príncipe VIII, Primoroso, Cartujano, Jamonero III, Innato, Mejicano, Habanero VIII, and Carocha III.

The marginal genetic contributions of the 15 most influential ancestors, founders or not, responsible for the presence of the breed in Brazil are shown in Table 4 for TP and RP. The ancestors that increased their marginal contributions in RP were Agareno, Príncipe VIII, Afiançado de Flandes, Estribilho, Whisky, Bailador, Quimono, and Sultão I. Ancestors Cartujano and Viscaina were replaced in RP by Hucharia and Guizo.

4. Discussion

4.1. Data analysis and distribution of birth records

In RP, only 0.2% of the animals were imported, indicating the sustainability of Brazilian breeding. These findings show that animals born in Brazil have the capacity to influence the future of the Lusitano breed in the world, with the possibility of Brazilian breeders becoming an exporter of Lusitano horses.

Similar onsets of birth records have been reported in the literature for the Brazilian Campolina [17] and Mangalarga Marchador [31] horse breeds. A decline in the registration of births was observed in the 1990s for the Brazilian horse breeds Mangalarga [16], Campolina [17], Brazilian Sport Horse [18] and Mangalarga Marchador [31]. According to the last authors, the reduction in the number of registered births can be explained by the implementation of the economic plan of the Brazilian government that resulted in a cause-effect relationship due to the oscillations in the Brazilian economy. In contrast, the annual number of registered births increased in the Lusitano breed during this period (1990s) (Figure 1), possibly because it is not a native Brazilian breed and was in a phase of importation and expansion in the country during that phase. According to the Anualpec yearbook [32], the number of horses decreased between 2002 and 2009 in Brazil, with a reduction of 278,375 births, while the number of Lusitano horses was stable during this period. The decline that started in 2010 (Figure 1) was the result of the serious economic-financial crisis in Europe and the United States, which caused a deceleration in horse breeding after 2008 in various parts of the world. A similar trend has been described in studies on the Lusitano [2], Old Kladruber [14] and Pantaneiro [15] breeds. The Lusitano breed in Brazil exhibited resistance to the decrease in equine records more than once, but the downward trend that started in 2010 (Figure 1) has become a matter of concern for the evolution of the breed. However, it is possible that some of the animals born in 2012 had not yet appeared in the database when it was consulted for the present study.

The importance of the month of birth is related to the fact that July is considered the ideal month for the birth of sporting animals in the southern hemisphere. Competitions start in July, i.e., animals born in this month have a competitive advantage over animals born in subsequent months because they are older and therefore exhibit greater physiological and sport development. This applies particularly to the classes of younger animals. Studies involving animals born in the southern hemisphere indicated the same trend observed for Lusitano horses registered in Brazil for the Mangalarga Marchador breed [31], with a concentration of birth (> 85%) between September and March. In the Mangalarga breed [16], a peak was found in November (17.4%) and a higher concentration between September and January (78.5%). A better but far from desired distribution was observed in Thoroughbreds [33], with 20.2% of births in the ideal month (July) and a larger number of births in August (24.1%), followed by September (23.0%). The distribution of births over the year in Brazil can be explained by the abundance of feed (pastures) throughout all quarters of the year and by the higher nutritional value in the last quarter (spring), a fact that leads Brazilian breeders to opt for the quality of their pastures at the expense of the ideal month. Since the Lusitano horse is mainly a sporting animal, the best choice is that a larger number of births should occur in the third quarter of the year. To help comparing the results and their discussion with several different horse breeds a supplementary table can be consulted online (Supplementary material - Table A).

4.2. Reproductive parameters and Generation interval

The differentiated values of breeding animals that left descendants (progenies) by national and imported origins are also observed in the literature. In the study of Cervantes et al [12] on Spanish Arab Horses, the contribution of imported individuals was high, corresponding to 47.9% of all descendants of the breed's studbook. Koenen et al [34], providing an overview of the breeding objectives for sport horses, reported percentages of mares covered by an imported stallion of 74% for Danish Warmblood, 62% for Swedish Warmblood, 32% for Irish Horse Board, 31% for Royal Dutch Sport Horse, and 6% for Selle Français. The percentage of use of imported Lusitano breeding animals among those imported to Brazil can be explained by the choices of breeders who purchased these animals from foreign populations mainly for breeding, with dams being used almost exclusively for this purpose. Despite the greater utilization within the origin of imported animals, the percentage of offspring with Lusitano parents born in Brazil was higher, demonstrating the reproductive sustainability on the American continent of the responsibility of Brazilian horse farms.

The percentages of breeding animals that left descendants are 3.3% (stallions) and 22.6% (mares) for the Mangalarga Marchador breed [31], and 8.3% (stallions) and 22.2% (mares) for the world Lusitano population [3]. These values are lower than those obtained in the present study, indicating greater utilization of breeding animals in the formation of the Lusitano population in Brazil and consequent higher genetic diversity. The smaller number of stallions in all studies reported may be due in part to the greater difficulty of approval in the studbook and their reproductive superiority, in which a single stallion can have dozens of offspring while a natural limit exists for mares. The low breeding stallion-to-mare ratio reduces the intensity of selection, which may result in less genetic progress of the population [35].

For the world Lusitano population, Vicente et al [2] indicated that the ratio of 5 mares per sire (lower than in the present study) impairs selection of the Lusitano breed. The small number of mares per stallions observed in the present study, similar to Quarter Horse in Brazil [19], should be taken into consideration by the association and by breeders, which can be modified and improved, increasing genetic progress to the levels (mares per stallion) of other breeds such as Thoroughbreds in New Zealand (43 to 1) [36] and Hanoverians (45 to 1) [11].

Vicente et al [2] reported mean numbers of offspring similar to those of the present study (Table 1) for the Lusitano breed, with a mean number of offspring of 13.1 for stallions and of 4.0 for mares, suggesting identical reproductive guidelines on Brazilian horse farms and in the remaining countries where Lusitanos are reared. The numbers of offspring observed in the literature were different (stallions) and similar (mares) compared to those of the Lusitano population in Brazil (Table 1), with 22.2 (stallions) and 3.1 (mares) offspring for the Campolina breed [17],10.7 (stallions) and 3.7 (mares) offspring for the Quarter Horse [19], and 23.8 (stallions) and 4.4 (mares) offspring for the Mangalarga breed [16]. Reproductive inequalities between sexes in horses can be seen in all breeds (Supplementary material - Table A), indicating greater participation of dams in the maintenance of the genetic diversity of each breed.

The age at birth of the first foal indicates the onset of reproductive life and, considering that horses are able to breed at 2 years of age [37], this did not influence the age at first foal since horses exhibit a late mean age at birth of their first progeny [2,38]. The main causes of this event as reported in various studies include differences in reproductive strategies between breeds and farms (high standard deviations in the results of Table 1), after functional performance and credits established in their sports career. Another factor are the regulations of the associations, which only permit the registry of animals in the studbook at certain ages and riding classes, a fact resulting in the approval of animals at older ages. Other factors include hormonal disorders, genital and parasite infections, and inadequate management practices before the breeding season, interfering with the onset of reproductive life of horses. An almost identical mean age at first progeny (Table 1) was reported for stallions of the world Lusitano population [2], with a difference of only 0.3 years. The delay in the age at first progeny in the Lusitano breed may be explained by the regulations of their association, which only permit the application of stallions after 4 years of age and of recommended or merit stallions (animals recommended by APSL for breeding can have an unlimited number of offspring) at a minimum age of 6 years. Many breeders who wish their mares covered by these stallions wait to perform matings or use the semen of these animals, consequently increasing the age at birth of the first foal. However, the mean age of mares (Table 1) of the present study was not far from the ideal proposed by Davies Morel et al [39]. These authors described the ideal age of mares to start breeding to be between 5 and 6 years, a period when they will have reached the final mature size.

The age at birth of the last progeny (Table 1) was similar to that reported by Vicente et al [2], who estimated a mean age of 10.5 years for sires and dams. The end of reproductive life of horses has been little studied and the age at last progeny is less reported than the age at first progeny. Further studies are necessary to understand the reasons for at age last progeny.

The coefficients of variation for all reproductive parameter indicate high dispersion of the results among animals, which was greater in stallions for all parameters, demonstrating an imbalance in the utilization of males and females. The results suggest different objectives within the Lusitano breed in Brazil, with the observation of sires and dams with a small or large number of offspring, late age at first progeny, and a reduced mean number of dams per sire. The values observed are common among some equine breeds, but are in contrast to the balanced values of cattle for which studies have already determined, for example, the ideal number of cows per bull [40].

Scientific studies have not shown the same influence on horse breeds as studies involving other livestock species (e.g., cattle, pigs, and small ruminants). The difficulty in designing the same experiments is mainly due to the peculiarities of horses and to the fact that, unlike in the case of other livestock species, the access of the scientific community to horse farms is limited, impairing the approximation between researchers and breeders. Breeders of the Lusitano horse in Brazil should increase and balance the number of dams per sire (genetic evolution), begin the reproductive life of animals earlier (decrease in GI), and collaborate more actively with researchers to establish new selection programs, which would permit to observe reproductive parameters that contribute to the genetic evolution of horses as observed for other livestock species.

Vicente et al [2], evaluating Lusitano horses using data collected throughout the world, obtained a GI that was similar to (TP) and the same as (RP) that of Lusitano in Brazil, indicating a possible influence of animals born in Brazil on the values of the world population. Although long, the GI is similar for all breeds in Brazil, such as Mangalarga (9.5 years) [16], Campolina (8.7 years) [17], Brazilian Sport Horse (10.8) [18] and Quarter Horse (9.6 years) [19]. The GI obtained in Europe for Andalusian (10.1 years) [8], Holstein (10.3 years) [9] and Old Kladruber (11.3 years) [14] was the same as those estimated for the Lusitano horse (Supplementary material - Table A) . A long GI is the result of different factors such as the late selection of stallions and mares and long reproductive life. Consequently, since the GI is present in the denominator of the formula used to calculate genetic gain, it will act inversely proportional, reducing the expected response to selection. The reduction in genetic gain due to a long GI in the Lusitano breed is supported by the high standard deviations, indicating that some breeders are concerned about the GI of their populations, while a large number of breeders who are not concerned are responsible for decelerating the evolution of the Lusitano breed in Brazil.

4.3. Quality of pedigree data

The mean values of pedigree completeness (Figure 2) tended to increase over time and the loss of data was lower when compared to a study on Spanish Arab horses [12], which reported percentages of 92.0%, 86.6% and 80.8% for the three most recent generations. For Lipizzan [40] and Old Kladruber [14], the authors indicated high values of completeness around 100% for the last five and six generations, respectively. In the study of the world Lusitano population, the percentages of pedigree completeness obtained for the whole population of 98.8%, 97.8% and 97.1% [2] indicate that the APSL data are slightly more complete than the whole data of ABPSL, with more in-depth pedigree knowledge. The estimation of pedigree completeness is important since the parameters related to the inbreeding coefficient, probability of gene origin and genetic drift of a given individual depends on how much of his ancestry is known. The moderate values of the present study can be improved, the greater this knowledge, the more reliable will be the estimated values in relation to the base population studied.

When compared to the literature, the number of GE (5.7 in RP) can be considered low, but was higher than that reported in studies on Pantaneiro (0.7 GE) [15], Brazilian Sport Horse (1.7 GE) [18], Paint-Horse (4.8 GE) [10], Quarter Horse (5.1) [19] and equal to Holstein (5.6 GE) [9]. However, the GE was lower than in Purebred Lusitano (9.9 GE) [2] and Old Kladruber (15.1 GE) [14]. Similar to the literature (Supplementary material - Table A), the increase in the amount of pedigree data in RP indicates improvement in the quality of pedigree information in the studbook of ABPSL. However, we must always analyze and compare these results with care since they depend on the quality and degree of completion of the pedigrees, so the values obtained for GE may have a direct influence on inbreeding, fe and fa estimates.

4.4. Parameters related to the inbreeding coefficient

The main effect of F (Table 3) is an increase in homozygosity as a result of more closely related animals. A higher inbreeding coefficient (9.4%) has been reported by Vicente et al [2] for the whole population of Lusitano horses registered in the world when a period of 5 years (2005 to 2009) was analyzed. An even higher value (11.3%) was obtained by the authors for a reference population. An inbreeding coefficient (F) of 4.6% was observed exclusively for Lusitano born in Brazil and for the same period (2005 to 2009). Considering the values reported by Vicente et al [2], Lusitano horses born in Brazil contribute to an overall decrease of inbreeding in the breed. This fact may indicate that farms have been introducing different lineages of less related Lusitano horses in Brazil over the years. On the other hand, this reduction in the inbreeding coefficient of animals reared in Brazil may also be explained in part by the lower level of pedigree knowledge compared to the Lusitano studbook of APSL. The maintenance of a low level of inbreeding in Brazilian Lusitano horses may contribute in the future to the export of breeding animals in order to reduce inbreeding on Lusitano horse farms around the world. Other authors reported higher value of F for Andalusian (8.5%) [8] Lipizzaner (10.8%) [13] and Old Kladruber (13.0%) [14]. Low levels of F are observed for Brazilian breeds, including Campolina (1.3%) [17], Brazilian Sport Horse (0.24%) [18] and Mangalarga Marchador (5.7%) [31] (Supplementary material - Table A). Low levels of F are an indicator that the breeds are organized in an open studbook system, which permits the utilization of sires and dams from other breeds with a consequent reduction in inbreeding.

ΔF was reduced (Table 3), with a small decrease in RP, despite the observation of a larger number of mating among related animals compared to TP. An increase in consanguinity was observed among annual births, finishing 2012 with 99.5% of births of related animals. The standard deviations obtained (Table 3) are not of major concern since they remained within acceptable levels for inbreeding parameters. High standard deviations were also observed in the study of Vicente et al [2]. Despite the lower dispersions in the present study, the results should not be overlooked because the small number of Lusitano horses compared to other breeds or species may, at any time, change the F values to less desired levels.

Values of N_e have been reported for Hanoverian (372) [11], Spanish Sport Horse (226) [41], Brazilian Sport Horse (223) [18], Holstein (55) [9], and Old Kladruber (53) [14] breeds. Studies that obtained high values of N_e suggest, by definition, reduced inbreeding levels because of the direct relationship between N_e and inbreeding rate [24]. This is obviously also related to the fact that most of these breeds have open studbooks, thus permitting the introduction of new genes and a consequent reduction in inbreeding. The small increase of N_e in RP (Table 3) is related to the fact that the increase in inbreeding was lower in the last generation, and possibly to the introduction of new lineages and different utilizations of major ancestors, either founders or not (Figure 3; Table 4). However, and according to Caballero and Toro [42], the comparison of different N_e values is problematic since the true value is unknown and the N_e obtained depends in part on the effect of changes in mating policies (level of inbred animals).

For the world Lusitano population, Vicente et al [2] reported that the N_e of 41 obtained for a period of 5 years (2005 to 2009) is a matter of concern for the maintenance of genetic diversity. The N_e observed in the present study for the same period and using the same method as those authors (individual increase in inbreeding) was 57. Considering the minimum N_e (50) recommended by the FAO [43], the value obtained in this study is less worrisome for the maintenance of genetic diversity, and Lusitano horses born in Brazil may therefore assist in maintaining the diversity of the world population.

4.5. Probability of gene origin and genetic drift

Lower probabilities of gene origin are reported in the literature for the Lusitano breeds (f_e of 28, f_a of 12 and f_g of 6) [2], Holstein (f_e of 50, f_a of 29 and f_g of 17) [9] and Old Kladruber (f_e of 93, f_a of 17 and f_g of 5) [14] and, while higher values were found for Paint-Horse (f_e of 561, f_a of 208 and f_g of 139) [10], Brazilian Sport Horse (f_e of 466, f_a of 274 and f_g of 224) [18], Quarter Horse (f_e of 1,045, f_a of 156 and f_g of 105) [19] and Spanish Sport Horse (f_e of 963, f_a of 407 and f_g of 254) [41] (Supplementary material - Table A). Higher values suggest the use of a larger number of animals in the formation of the breeds. The genetic variability of breeds observed in the literature, as demonstrated by the results of the probability of gene origin, is a matter of concern in horse breeding. However, the variation in the three parameters of gene origin probability obtained in the present study was lower than that described in the literature. The effective number of founders in both populations, comparing the number of founders (Table 3), indicates the preferential use of certain lineages of founders. Since each population has its own characteristics, comparison with the literature should be done with caution and within each breed, considering the three parameters for each population.

Genetic drift is approaching stability in the Brazilian Lusitano breed (Table 3), with a progressive increase in the representation of founders [28,29] in RP as indicated by $f_e/N_e > 0.5$ and $f_e \sim (N_e/2)$, which are close to the desired. The f_e/f_a ratio (Table 3) confirms the absence of worrisome bottleneck effects in the population despite the increase of 0.02 in RP. Different results are observed in the literature, with the loss of genetic variability due to the utilization of lineages with a reduced number of founders and worrisome bottleneck effects; for example, f_e/f_a ratios of 1.69 for the Brazilian Sport Horse [18], 1.72 for the Holstein [9], 2.34 for Lusitano using world data [2], 2.37 for the Spanish Sport Horse [41], 2.70 for the Paint-Horse [10], 5.47 for the Old Kladruber [14], and Quarter Horse 6.70 [19].

. The persistence of the founder lines is observed in several breeds throughout the world, being lower in the present study. It should be noted that, the values of each breed may vary due to different qualities of Studbook data information (Pedigree completeness and GE).

4.6. Genetic conservation index

In the literature, mean GCIs of 1.3, 9.5 and 14.8 and maximum values of 7.2, 19.2 and 31.2 have been reported for the Pantaneiro [15] Lusitano [2] and Quarter Horse [19] breeds, respectively (Supplementary material - Table A). The small differences between the world Lusitano population and the present study are visible in the maximum values and are explained by the heterogeneous importation of genetic lineages of Lusitano into Brazil. Consequently, different sires and dams were used over time, which resulted in slightly higher GCI values.

Animals with higher indexes exhibit greater conservation, i.e., a greater balance in the number of founders, and should be used (preferentially in the choice of breeding animals) in genetic selection programs to maintain the genes transmitted by founders [30]. Brazilian farms are a good choice for breeding animals of the Lusitano breed or of world breeds with origin in the Lusitano horse, with the Mangalarga Marchador, Campolina, Andaluz Brasileiro or Iberian breeds (e.g. Portuguese and Spanish Sport Horses), in order to introduce founder genes of the Lusitano breed.

4.7. Genetic contributions

A slight increase in the genetic contributions explained by the 10, 50 and 100 most influential founders was observed from TP to RP (Table 3), indicating a similar use of lineages as the major founders in RP. This change contributed to maintaining the genetic diversity of the Lusitano breed in Brazil over the last 10 years (2003-2012) evaluated. The marginal genetic contributions of ancestors, with a small increase in RP (Table 3), suggest that ancestors contributed equally in TP and RP, demonstrating the maintenance of alleles of ancestral sires and dams in the last generation.

The marginal genetic contribution of the 15 most influential ancestors in the present study (Table 4) was higher than that reported for Hanoverians (34.9%) [11]. Considering the 10 most influential ancestors with the greatest marginal genetic contributions to the world Lusitano population reported in the study of Vicente et al [2], half of these animals were also ancestors of the Brazilian population (Table 4), represented by Agareno, Príncipe VIII, Destinado IV, Primoroso and Cartujano, at different proportions and maintaining only the order of the marginal genetic contribution of the most influential ancestor (Agareno). Although these authors studied the same breed as in the present study, the data used refer to the period from 2005 to 2009, with a much larger sample of data and the approaches taking place in different populations. Consequently, the observation of differences in the parameters between the two studies was expected.

In the study on the world Lusitano population [2], the number of founders was 14 and 99, corresponding to 50% and 90% of the genetic contribution of founders, respectively. The Brazilian Lusitano population exhibited variations in founder lineages (Figure 3) and, consequently, greater genetic diversity of the animals born on Brazilian farms. These differences may help explain the lower inbreeding and greater genetic diversity of Lusitano horses born in Brazil.

5. Conclusion

The inbreeding and relatedness coefficients demonstrate some control on the part of Brazilian breeders, suggesting that Lusitano horses born in Brazil can be introduced as breeding animals in the world Lusitano population in order to increase the genetic diversity of the breed. The long GI obtained indicates a delay in annual genetic gain and the lack of speedy decision-making about the appropriate use of sires and dams. Although the absence of bottleneck effects is evident and positive, the low probabilities of gene origin and the high genetic contribution of a small number of animals suggest that Brazilian breeders need to monitor the genetic diversity of the Lusitano horse in next generations through breeding programs.

Acknowledgments

The authors thank the Associação Brasileira de Criadores do Cavalo Puro-Sangue Lusitano (ABPSL) for providing the data used in this study. We are also grateful to the anonymous reviewers for their helpful comments. We acknowledge the invaluable contribution of the late Professor Dr. Marcílio Dias Silveira da Mota.

Funding

Productivity grant attributed to Professor Dr. J. Augusto II, by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - Brazil).

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Figure captions

Figure 1. Number of births (males, females, and all animals) registered in the Studbook of the Lusitano horse breed in Brazil by year of birth.

Figure 2. Completeness of pedigree information per generation for both populations (total and reference) of Lusitano horses in Brazil (15th generation of older animals).

Figure 3. Evolution of the genetic contribution of the 10 most influential founders of the Lusitano breed in Brazil from the first record in 1967 to 2012, during five different periods.

Tables

Table 1. Summary descriptive statistics of reproductive parameters of Lusitano stallions and mares in Brazil

	Total population	
Item	Stallion	Mare
Offspring		
Total (n)	18,515[a]	18,436[b]
Mean ± SD (n)	12.3 ± 24.5	4.0 ± 3.4
Maximum (n)	419	20
Stallions/mares		
Total (n)	1,507	4,644
Age at first progeny (years)	7.0 ± 3.8	5.9 ± 3.1
Age at last progeny (years)[c]	10.7 ± 5.7	10.5 ± 5.3

N number of the respective observation; SD, standard deviation. [a] offspring with known father (independently of knowing the mothers). [b] offspring with known mother (independently of knowing the father). [c] Only calculated for animals with two or more offspring.

Table 2. Generation interval (in years) for the four paths of selection between parents and offspring that reproduced for Lusitano horses in Brazil

Path of selection	Total population		Reference population	
	n	Mean ± SD	n	Mean ± SD
Father-son	1,393	10.9 ± 6.0	146	9.4 ± 4.7
Father-daughter	4,409	10.8 ± 5.5	679	11.4 ± 6.0
Mother-son	1,374	9.5 ± 4.5	144	10.2 ± 4.7
Mother-daughter	4,350	9.2 ± 4.5	679	9.6 ± 4.4
Overall mean	11,526	10.1 ± 5.1	1,648	10.2 ± 5.0

n number of observations; SD standard deviation.

Table 3. Summary statistics of parameters related to inbreeding, probability of gene origin, genetic drift and genetic contributions of founders and ancestors of the Lusitano breed in Brazil

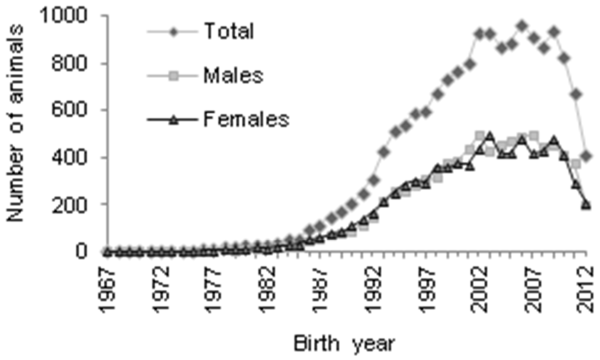
Item	Total population	Reference population
Parameters related to inbreeding		
Average inbreeding coefficient[a], F (%)	4.06 ± 4.94	4.46 ± 4.34
Average related coefficient, AR (%)	5.41 ± 1.69	5.97 ± 1.20
Increase in inbreeding[a], ΔF (%)	0.96 ± 2.09	0.85 ± 0.90
Effective population size[b], N_e (n)	89	90
Effective population size[c], N_e (n)	52	59
Number of animals with inbreeding coefficient different from zero (n)	16,777	8,160
Probability of gene origin		
Effective number of founders, f_e (n)	33	29
Effective number of ancestors, f_a (n)	30	26
Effective number of founder genomes, f_g (n)	19	15
Genetic drift		
f_e / N_e[c]	0.64	0.49
f_e / f_a	1.10	1.12
f_e ~ (N_e / 2)[c]	33 ~ 26	29 ~ 30
Contributions of founders and ancestors		
Total number of animals per population	18,922[d]	8,329[d]
Total number of animals with both parents known (n)	18,406[e]	8,315[e]
Number of founders (n)	516	453
Genetic contributions of the 10 most influential founders (%)	42.7	43.5
Genetic contributions of the 50 most influential founders (%)	74.3	78.2
Genetic contributions of the 100 most influential founders (%)	85.7	88.7
Number of ancestors (n)	427	341
Genetic contributions[d] of the 10 most influential ancestors (%)	46.8	52.2
Genetic contributions[d] of the 50 most influential ancestors (%)	82.0	84.6
Genetic contributions[d] of the 100 most influential ancestors (%)	91.7	93.1

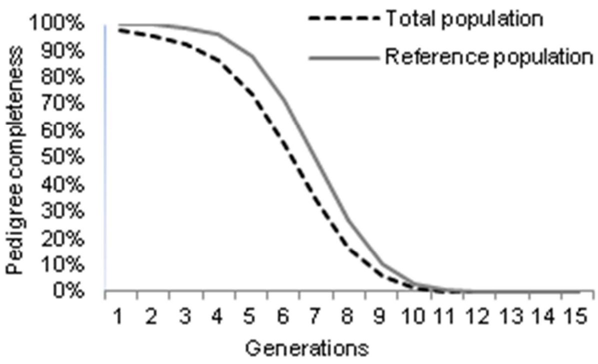
[a] All animals in the population studied. [b] According to Falconer and Mackay (1996). [c] According to Gutiérrez et al. (2009). [d] total animal studies in each population. [e] total of animals with known father and mother in each population. [f] Marginal genetic contributions.

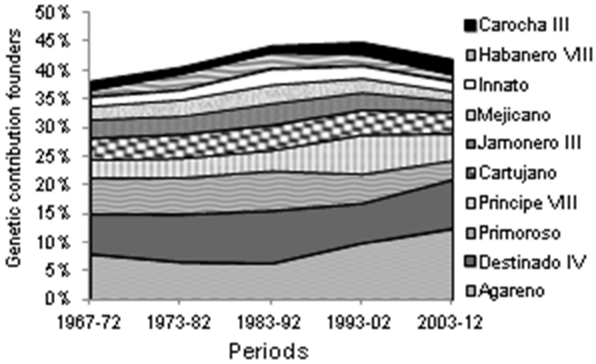
Table 4. Marginal genetic contributions (in %) of the 15 most influential ancestors (founders or not) to the Lusitano breed in Brazil

No.	Name	Year of birth	Sex	Breeder	Marginal contribution (%)
Total population					
1	Agareno	1931	S	Manuel Tavares Veiga	10.62
2	Príncipe VIII	1943	S	D. Francisco C Navarro	7.12
3	Destinado IV	1940	S	D. Francisco C Navarro	6.35
4	Primoroso	1927	S	Hermanos Dominguez	4.61
5	Cartujano	1928	S	D. António Perez Tinao	4.09
6	Bailador	1962	S	Manuel Tavares Veiga	3.20
7	Estribilho	1963	S	M Assunção Coimbra	2.85
8	Jamonero III	1953	S	D. Isabel M V Terry	2.70
9	Viscaina	1933	M	D. M Romero Benitez	2.64
10	Afiançado de Flandes	1982	S	Quinta de Flandes	2.60
11	Whisky	1947	M	F Sommer D'Andrade	2.38
12	Babel	1965	S	L J Ortigão Costa	2.32
13	Quimono	1974	S	A J Fonseca Alcobia	2.23
14	Innato	1962	S	D. J Domeq de La Riva	1.94
15	Sultão I	1942	S	Manuel Tavares Veiga	1.78
All 15					57.45
Reference population					
1	Agareno	1931	S	Manuel Tavares Veiga	12.48
2	Principe VIII	1943	S	D. Francisco C Navarro	8.58
3	Destinado IV	1940	S	D. Francisco C Navarro	4.73
4	Afiançado de Flandes	1982	S	Quinta de Flandes	4.20
5	Estribilho	1963	S	M. Assunção Coimbra	3.85
6	Whisky	1947	M	F Sommer D'Andrade	3.84
7	Bailador	1962	S	Manuel Tavares Veiga	3.44
8	Primoroso	1927	S	Hermanos Dominguez	3.33
9	Quimono	1974	S	A J Fonseca Alcobia	2.95
10	Hucharia	1943	M	Estado Português CN	2.74
11	Babel	1965	S	L J Ortigão Costa	2.04
12	Sultão I	1942	S	Manuel Tavares Veiga	2.04
13	Jamonero III	1953	S	D. Isabel M, V Terry	2.00
14	Innato	1962	S	D. J Domeq de La Riva	1.69
15	Guizo	1947	S	Manuel Tavares Veiga	1.69
All 15					59.87

No. number; S Stallion; M mare.


Total
Males
Females
Number of animals
Birth year
1000
800
600
400
200
0
1967
1972
1977
1982
1987
1992
1997
2002
2007
2012


Total population
Reference population
Pedigree completeness
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Generations



50 %
45 %
40 %
35 %
30 %
25 %
20 %
15 %
10 %
5 %
0 %
Genetic contribution founders
1967-72
1973-82
1983-92
1993-02
2003-12
Periods
Carocha III
Habanero VIII
Innato
Mejicano
Jamonero III
Cartujano
Principe VIII
Primoroso
Destinado IV
Agareno

Highlights

- The Brazilian Lusitano horse has the capacity to influence the future of the Lusitano breed in the world.
- The low level of inbreeding in Brazilian Lusitano represents an export market for breeding animals.
- Maintenance of genetic diversity in the last population.
- Absence of bottleneck effects and genetic stability of the Lusitano breed in Brazil.