Evaluation of conservation program for the Pantaneiro horse in Brazil

Concepta McManus[1,2], Sandra Aparecida Santos[3], Bruno Stéfano Lima Dallago[1], Samuel Rezende Paiva[4], Rafhael Felipe Saraiva Martins[1], José Braccini Neto[2], Pedro Rocha Marques[2], Urbano Gomes Pinto de Abreu[3]

[1] Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília, Brasília, DF, Brazil, 70910-900.
[2] Department of Animal Production, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, 91540-000.
[3] EMBRAPA Pantanal, Caixa Postal 109, Corumbá, MS, Brazil, 79320-900.
[4] EMBRAPA Recursos Genéticos e Biotecnologia, Final W5 Norte, Brasília, DF, Brazil, 70770-917.

ABSTRACT - A genealogical analysis of registered Pantaneiro horses was carried out with 3647 males and 6794 females. Of these animals, 50.66% had identified sires and 48.86% identified dams. The number of pedigrees increased over the generations, with higher registration of parents of sires than dams. Two municipalities are responsible for almost 70% of all registers: i) Poconé (Mato Grosso State), where the headquarters of the breeders association is located and ii) Corumbá (Mato Grosso do Sul State), where EMBRAPA Pantanal conducts its research. The mean inbreeding was 0.04% and average relatedness was 0.13%. Mean generation interval was 8.20 years. There is moderate to high genetic differentiation between farms (15% of total genetic variation) while between municipalities there is little differentiation. Wright´s fixation statistics were calculated and F_{IS} (inbreeding coefficient of individuals relative to the subpopulation) values indicate some heterozygosity between farms but not municipalities, with overall F_{IT} (inbreeding coefficient of individuals relative to the total population) close to zero. The municipalities with the highest number of animals (Poconé and Corumbá) import relatively fewer sires (45 and 67% respectively). Genetic indices from genealogical data on the Pantaneiro horse population show that inbreeding is under control. Future breeding plans should include germplasm exchange between municipalities. The conservation program for the Pantaneiro horse has been shown to be successful but careful planning is needed in the future to avoid inbreeding and changes in important breed traits given the increasing interest in the use of the breed in sporting competitions.

Key Words: average relatedness, effective population size, farm, inbreeding, municipality, Wright´s statistics

Introduction

The Pantanal occupies 250,000 km², of which 138,183 km² are in Midwest Brazil (1.76 per cent of the total Brazilian territory). The rest of the area is in Bolivia and Paraguay. It is the largest freshwater wetland of the world, a seasonally flooded plain fed by the tributaries of the Paraguay River, with 65 per cent of its area in Mato Grosso do Sul (MS) State and 35 per cent in Mato Grosso (MT) State (UNEP, 2007).

The Pantaneiro is a naturalized horse breed from the Pantanal region of Brazil. This horse probably originated from Iberian horses introduced by Spanish settlers, especially in the 16th and 17th centuries, and by Portuguese settlers in the 18th century (Santos et al., 1992). As a consequence of natural selection for more than two centuries, with little or no human interference, this breed became well adapted to the environment. At the end of the 19th century, the population was severely reduced due to *Peste das cadeiras* (*Trypanossomiasis*).Later, other menaces appeared, mainly indiscriminate crossbreeding and more recently Equine Infectious Anemia (EIA). This genetic resource was saved from extinction by the creation of the Brazilian Association of Pantaneiro Horse Breeders (ABCCP), in 1972, using a base herd of 100 animals (92 mares and 8 stallions). These were distributed in four nuclei (three in Poconé municipality and one in Cáceres, both in MT). There are currently about 160 breeders, in various subregions. Most of them are in MT, in the High Paraguay River Basin, expanding into Bolivia and Paraguay (Santos et al., 2003).

The main use of this horse is to manage cattle, which is the principal economic activity in the region. The cattle are driven on foot by mounted cowboys and the journeys across the flooded areas can last weeks as there are few or no roads (Abreu et al., 2010). More recently, the breeders have become interested in selection and improvement of the breed, principally in its body conformation and use in tournaments and rodeos. Although conformation is related to performance, selection should be directed to make sure that the breed does not lose its traits attained through natural selection (McManus et al., 2008). According to Valera et al. (2005) the assessment of within-population genetic variability and gene flow is necessary before

undertaking selection programs. This is to establish appropriate management of genetic stock as management policies may have a large impact on genetic variability. A growing interest in the Pantaneiro horse has been seen in the last decade, not only because of its functionality but also because of its high market value.

The demand for descendants of famous stallions is very high, so there is a need to know the within-population genetic variability before the implementation of selection and conservation programs. Therefore, the objective of this study was to evaluate the conservation program using the genealogical register for the breed, aiming to develop strategies to control inbreeding and maintain genetic variability to aid in conservation. The information will also be used to develop selection programs, as well as evaluate the effect of recent selection strategies carried out by the breeders, which has been primarily based on conformation characteristics.

Material and Methods

The population studied was 10,441 animals which included all animals (5,359 definitive and 4,741 provisional) registered by ABCCP (Brazilian Association of Pantaneiro Horse Breeders) from its creation in 1972 to August 2009. There were 3,647 males and 6,794 females. A number of population and genetic parameters were computed for the reference populations. These included the animals with both parents known, and separated by farms and municipalities using the program ENDOG v4.8 (Gutiérrez & Goyache, 2005). The following parameters were calculated: the pedigree completeness level, computed as the proportion of ancestors known per parental generation (MacCluer et al., 1983); the number of equivalent to discrete generations (*t*) for each individual in a pedigree (Boichard et al., 1997); the individual inbreeding coefficient (*F*), computed according to Meuwissen & Luo (1992); and average relatedness coefficient (AR) (Goyache et al., 2003; Gutiérrez et al., 2003). The probability of gene origin was characterized by computing the following parameters: effective number of founders (f_e) (James, 1972), computed from the genetic contribution of founders to the descendant gene pool of the population (Lacy, 1989); effective number of ancestors (f_a), defined as the minimum number of ancestors, not necessarily founders, explaining the complete genetic diversity of a population (Boichard et al., 1997); founder genome equivalents (Ballou & Lacy, 1995), obtained by the inverse of twice the average coancestry of the individuals within the population (Caballero & Toro, 2000).

Effective population size (N_e) was computed in several manners and refers to the number of breeding animals that would lead to the actual increase in inbreeding if they contributed equally to the next generation ($N_e = 1/(2\Delta F)$). It was estimated by computing the regression coefficient of the individual inbreeding coefficient over: i) the number of full generations traced; ii) the maximum number of generations traced and iii) the equivalent complete generations. Effective population size was also estimated via individual increase in inbreeding (ΔF_i), as proposed by (Gutiérrez et al., 2008).

Wright's fixation (F) statistics were calculated assuming the population has a population structure of two levels; one from the individual (I) to the subpopulation (S) and another from the subpopulation to the total (T). These F-statistics describe the amount of inbreeding-like effects within subpopulations (F_{IS}), among subpopulations F_{ST}, and within the entire population (F_{IT}). In this case Wright's *F*- statistics are obtained according to Caballero & Toro (2000, 2002). These calculations were carried out on all records available and the reference population defined as all animals with parents identified (ALL) and on a subgroup of animals born since January 1998 (RECENT) to represent the present population.

The within-population coancestry (f_{ii}) and the between-population coancestry matrix (f_{ij}) were computed averaging all pairwise coancestry coefficients of the individuals belonging, respectively, to a given population *i* or to two different populations *i* and *j*. Following Caballero & Toro (2000, 2002), the between-population Nei's minimum distance (D_m) matrix was also computed as $D_m = (f_{ii} + f_{jj})/2)-f_{ij}$, where f_{ii} and f_{jj} are the average coancestry within two populations *i* and *j* and f_{ij} the coancestry between two populations *i* and *j*. In the present study, municipalities and farms were considered separate populations; the effective number of founders in an animal pedigree was calculated using the genetic conservation index, GCI (Alderson, 1992): $GCI = 1/\Sigma P_i^2$ where P_i is the proportion of genes of founder animal *i* in the pedigree.

Mean inbreeding per generation was used to form a regression equation testing both linear and quadratic functions. This was then used to predict further inbreeding up to 15 generations.

Results

Of all the animals registered, 50.66% had known sires and 48.86% known dams (Figure 1). For all generations, relatively more information was available for the male pedigree than the female, but in the last two generations increased information on females was available. The number of pedigrees known increased over the generations,

with higher registration of parents of sires than dams. This is particularly evident in the 4th (40% lower registration for dams) and 5th (96%) generations.

The number of offspring per sire (410 sires) ranged from one to 105, with a mean of 12.75, while for dams (2,226) it ranged from one to thirteen with a mean of 2.56.

The increase in number of registrations per year is evident (Figure 2). Two municipalities are responsible for almost 70% of all registers: i) Poconé, where the breeders association is located (*Latitude* 16º 15' 24" South and *Longitude* 56º 37' 22" West) and ii) Corumbá, where EMBRAPA Pantanal conducts its research on animals (Latitude 19º 00' 33" South and Longitude 57º 39' 12" West). The total number of founder municipalities in the ALL population (both parents known) was 32, with 3.4 effective founder municipalities, with 24 effective founder herds in the ALL population and 35.3 in the RECENT population.

The farms with the highest number of horses registered were Rancharia (758), Carandá (518), Promissão (477) and São José das Águas (403), all classified as multiplier herds. The conservation herd of EMBRAPA (Nhumirim) appears in 8th place, with 241 animals registered. Concerning the parameters for the two reference populations used (ALL and RECENT; Table 1), the effective number of founders (f_e) for ALL was 312 (30.8% of founder population) with 297 effective ancestors (f_a) for the ALL reference population (6.2%), while for the RECENT population they were 683 and 618, respectively. A total of 152 animals supplied 50% of the ancestors in ALL and 543 in RECENT.

For the RECENT population there were 5,772 animals registered. Because it included a large portion of animals without one or both parents known, it was larger than the ALL population. This also accounts for the larger effective number of founders and ancestors in the RECENT population.

Several other estimates of N_e are available (Table 1) depending on the method used for their calculation. The differences between these measures are a reflection of the different methods used and the number of animals used in each case, but estimates are relatively consistent between ALL and RECENT. The mean inbreeding was 0.04% and AR was 0.13%. As the generations increase, an increase in inbreeding is observed (Table 2). This is especially evident in generation 5, where there was almost a 14% increase in inbreeding. In this generation there were eight matings (0.99%) between sibs and ten (0.39%) between parents and off-spring. Four stallions had approximately 100 foals each, which also had the highest AR values (ranging from 1.26 to 0.69%). Six mares had ten or more foals. Effective

Table 1 - Summary statistics for reference populations analyzed in this study

	ALL	RECENT
Number of animals	10,441	
Mean inbreeding	0.04%	
Mean average relatedness	0.13%	
Base population (one or more unknown parents)	5,498	
Actual base population (one unknown parent = half founder)	5,110	
Effective population size of founders	964.28	
Expected inbreeding by unbalancing of founders	0.05%	
Computed mean inbreeding	0.04%	
Age becoming parent for the first time	5.48 years	
Regression coefficient of age becoming parent on inbreeding coefficient		12.20 (14.14)
Regression coefficient of age becoming parent on increase in inbreeding coefficient	25.80 (23.14)	
Number of animals in the populations studied[1]	4,900	5,772
Effective size obtained from regression on the birth date	1,978.54	613.65
Effective size obtained from log regression on the birth date	1,779.28	538.16
Effective number of founders (f_e)	312	683
Effective number of ancestors (f_a)	297	618
Number of ancestors explaining 50%	152	543
Number of founder herds	153	182
Effective number of founder herds (f_h)	24	35.3
Number of founders	2,171	3,331
Equivalent number of founders	2,122	3,180
Ancestors contributing to reference population	2,165	3,330
Ne due to individual increase in inbreeding	124.99(57.82)	125.36 (58.45)
Number of individuals	477	447
Individual increase in coancestry		237.21(8.34)
Effective Ne regression on equivalent generations	25.06 (0.91)	24.75 (0.88)
Effective Ne log regression on equivalent generations	22.77	22.5
Ne regression on birth date	53,47	51.13
Ne log regression on birth date	40.09	44.85

Ne - effective population size; in brackets - standard error.
[1]ALL - reference population of all animals with both parents known; RECENT - registered animals from January 1st, 1998, regardless of whether parents are known.

Table 2 - Inbreeding (F), average relatedness (AR) and effective population size (*Ne*) per generation in the Pantaneiro horse breed

Generation	Number of animals	Mean F (%)	%	Mean F for inbred (%)	% Mean AR	*Ne*
			Inbreeding per generation[1]			
1	2,851	0.00			0.15	
2	1,328	0.09	0.45	20.83	0.26	531.2
3	752	0.12	1.33	9.06	0.34	1,893.1
4	325	0.44	4.31	10.10	0.35	158.7
5	115	0.58	13.91	4.15	0.33	239.7
			Inbreeding per complete generation[2]			
1	4,328	0.03	0.23	14.69	0.21	1,473.3
2	324	0.85	11.11	7.68	0.42	60.9
3	1	0.00			0.32	

[1] Number of full generations traced.
[2] Maximum number of generations traced as defined by Gutiérrez et al. (2009).

population size calculated here refers to that calculated on the whole population per generation and varies widely because of the changes in the number of animals entering the population at different times.

Generation intervals (Table 3) are lower for dams than sires in both populations. This reflects the use of stallions for longer periods in the herds than the mares. RECENT generation intervals are longer than those found in ALL.

Inbreeding levels per generation (Figure 3) show a steady increase in inbreeding rates, although rates are still relatively low.

Although there were 37 years of data available, the number of fully traced, maximum and equivalent generations evaluated is still low (Table 4) reflecting the lack of pedigree information in the early years of the breeders association. The N_e calculated from them reflect the lower, upper and real limits of this parameter, respectively.
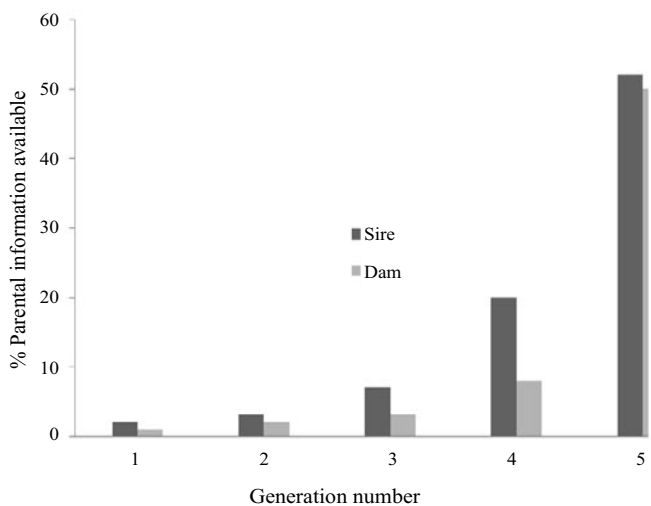

Figure 2 - Number of Pantaneiro horses registered per year of birth

Figure 1 - Percentage of pedigree known per generation of Pantaneiro horse.

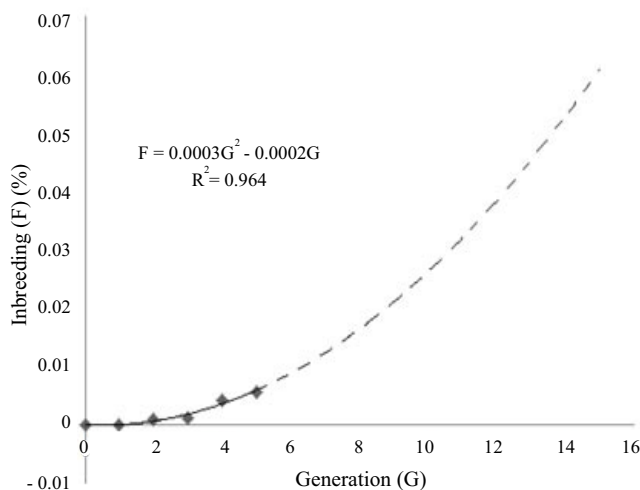$$F = 0.0003G^2 - 0.0002G$$
$$R^2 = 0.964$$

Figure 3 - Mean (1st to 5th generation) and predicted (6th to 15th) inbreeding in the Pantaneiro horse.

Table 3 - Number of animals (N), generation intervals (L) and standard errors (SE) for the Pantaneiro horse

Relationship	ALL			RECENT		
	N	L	SE	N	L	SE
	Generation intervals					
Father-son	151	8.75	0.37	73	9.55	0.57
Father-daughter	358	8.85	0.22	170	9.44	0.48
Mother-son	141	7.49	0.27	73	7.81	0.39
Mother-daughter	339	7.57	0.19	173	8.09	0.43
Total	989	8.20	0.13	489	8.74	0.18
	Mean age of parents when offspring born					
Father-son	2,860	9.42	0.09	1,851	10.19	0.11
Father-daughter	2,531	9.28	0.10	1,708	9.91	0.11
Mother-son	2,722	7.95	0.07	1,875	8.43	0.09
Mother-daughter	2,436	7.67	0.08	1,724	8.17	0.09
Total	10,549	8.60	0.04	7,158	9.18	0.5

In the present study when farms are considered subpopulations (Table 5) there is moderate to high genetic differentiation (15% of total genetic variation), while between municipalities there is little differentiation. Inbreeding levels were low. The F_{ST} values for municipalities and farms were similar, while F_{IS} values indicate some heterozygosity between farms but not municipalities with overall F_{IT} close to zero.

Careful mating strategies should be planned to avoid inbreeding (Figure 4). Of the farms studied, sixty had F_{ST} scores higher than 0.1 and 68% of these were in Poconé. Corumbá had the second highest level with 10% of the farms. This would suggest that special care should be taken when deciding which stallions to use in these municipalities, and one should avoid importing stallions from municipalities such as Campo Grande, Rio Verde, Cáceres and attempts should be made to use more sires from Barra do Bugres, Ladário and Rochedo.

Pantaneiro horse herds can be considered multiplier or commercial (Table 6), with no herds considered nucleus or isolated. Most herds (>91%) use foreign sires and more than 79% of municipalities import sires. This is on a numerical basis, but the municipalities with the highest number of animals (Poconé and Corumbá) import relatively fewer sires (45 and 67% respectively).

The herd structure shows that the number of herds registering Pantaneiro horses increases per generation, with present generation having 88 herds and almost 20 effective herds (Table 7).

Table 4 - Number of mean generations, inbreeding increase per generation and effective population size

	Mean generations	Increase in inbreeding (%)	Effective population size
Maximum	0.95	0.07	679.98
Complete	0.49	0.15	323.54
Equivalent	0.67	0.13	384.32

Table 5 - Genetic variation measures of the Pantaneiro horse breed among farms and municipalities

	Farm	Municipality
Mean coancestry within subpopulations	0.1539	0.0031
Selfcoancestry	0.5004	0.5002
Inbreeding	0.0008	0.0004
Nei Distance	0.1518	0.0025
Mean coancestry in the metapopulation	0.0020	0.0006
F_{IS}	-0.1809	- 0.0027
F_{ST}	0.1521	0.0025
F_{IT}	- 0.0012	- 0.0002

F_{IT} - inbreeding coefficient of individuals relative to the total population; F_{IS} - inbreeding coefficient of individuals relative to the subpopulation; F_{ST} - correlation between random gametes drawn from the subpopulation relative to the total population.
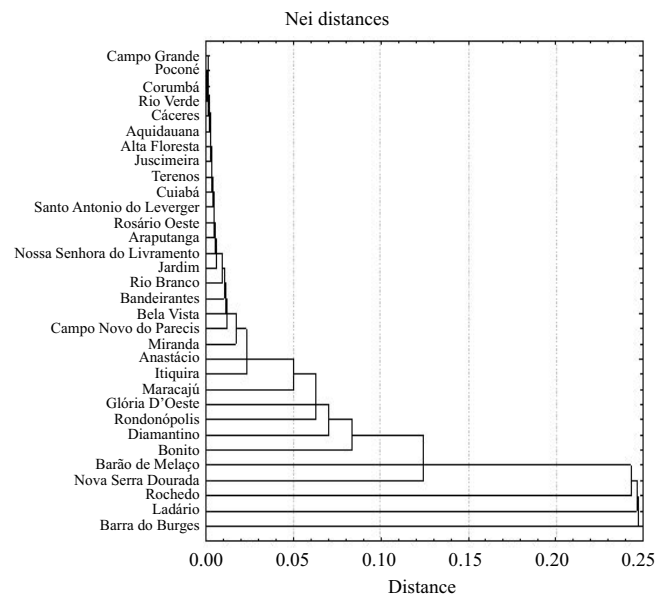

Figure 4 - Nei Distances between municipalities with registered Pantaneiro horses.

Table 6 - Definition of farm type of Pantaneiro horse

| | Definition | | | Herds | | Municipalities | |
	Using foreign sire	Using own sire	Selling aires	Number of herds	% Foreign sires	Number of municipalities	% Foreign sires
Nucleus	No	Yes	Yes	0	0	0	0
Multiplier	Yes	Yes	Yes	38	91.3	13	79.1
Multiplier	Yes	No	Yes	45	100	5	100
Commercial	Yes	Yes	No	4	97.3	0	0
Commercial	Yes	No	No	58	100	4	100
Isolated	No	Yes	Yes	0	0	0	0

Table 7 - Structure of herds per generation of the Pantaneiro horse

| Generation | Herds | | Municipalities | |
	Number	Effective number	Number	Effective number
Sire	5	102	24.24	18	3.07
Grand sire	4	37	12.58	10	2.29
Great (G) grand sire	3	15	9.10	5	1.70
GG grand sire	2	6	2.82	2	2.00
GGG grand sire	1	1	1	1	1

Mean GCI is 1.33 with the highest of 8.53 for a mare, with four mares and six stallions having a GCI of 7.16. Eight farms with a significant number of animals (>50) had mean GCI above 2, but the highest mean GCI was the Barra Mansa farm (2.68). These farms are distributed throughout the Pantanal in five different municipalities (Alta Floresta, Aquidauna, Poconé, Rosário Oeste and Corumbá), which is important for the conservation of the breed. The conservation nucleus for this breed on the Nhurumuim farm had a mean GCI of 1.82. Mean GCI for males was 2.10 and for females, 1.52. There was a significant increase in GCI over the years (Figure 5), with females lagging behind males until approximately 2005. Data from before 1980 was omitted because the mean for each year was 1.

Figure 5 - Genetic conservation index for Pantaneiro horses by year of birth.

Discussion

The study of genetic variability and the population structure in many equine breeds using pedigree analysis, alone or in association with genetic marker information, has increased in recent years (Cecchi et al., 2006; Poncet et al., 2006; Sabbioni et al., 2007).

The interest in horse populations compared with other species is probably due to homogenization of light breeds aimed at developing a type of horse for the practice of sports (Alderson, 1992) but in Brazil horses such as the Pantaneiro are mainly used for managing cattle in harsh environmental conditions (McManus et al., 2008).

As with other adapted animals in Brazil, this animal suffered risk of extinction in the 1970s. This was due to marketing the use of certain exotic species, which caused genetic erosion (Rege & Gibson, 2003). With the resurgence of the breed, it is now frequently used in rodeos and shows. This has also led to a more homogeneous population, since breeders preferentially use famous stallions because they do not want to risk losing a breed competition, thus probably limiting the available genetic variability in the breed.

The quantity of information on the Pantaneiro increased with generation number. This is in agreement with studies such as that of Valera et al. (2005) where more distant generations had less genealogical information as it may not have been recorded or was lost over the years. Pedigree data from horses in other countries tend to be more complete as studbooks have been kept for much longer (Cervantes et al., 2009).

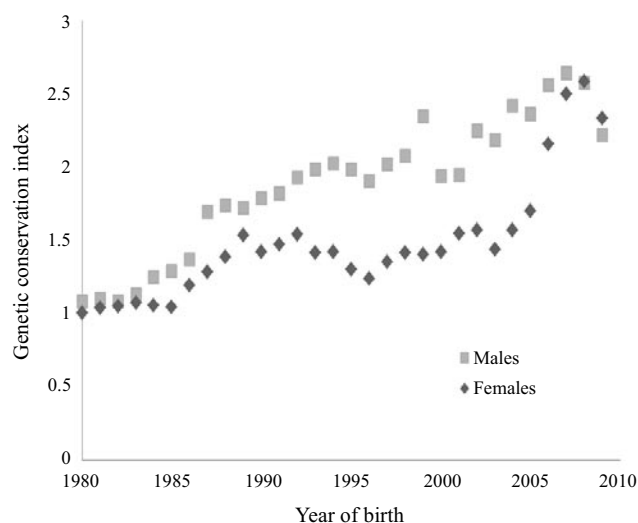The increase in number of registrations per year is evident (Figure 2), positively reflecting in the success

of the conservation program and increased interest in this horse. The fact that the largest populations are seen where the Breeders Association and EMBRAPA are located shows the importance of these institutions in the continued success of the conservation program. The effect of municipality is important, as farmers living close to each other may exchange, share or loan stallions thereby causing regional inbreeding, especially those beginning to breed the Pantaneiro horse or with few animals.

Genetic diversity in the Pantaneiro population is demonstrated by its estimation of number of ancestors, f_e, f_a and number of ancestors explaining 50% of genetic variation. According to Boichard et al. (1997), such parameters are less sensitive to pedigree length than inbreeding coefficients and N_e estimated by increase in inbreeding. The pattern observed in each estimate, when ALL and RECENT are compared, indicate that founder alleles are not being lost. This is the opposite of that found in Trakehner (Teegan et al., 2009) and Selle Français (Dubois & Ricard, 2007), comparing two periods for each breed. Other evidence of lack of allelic loss is revealed when comparing differences between parameters f_e and f_a in both moments. The ratio between them (f_e/f_a) is equal to 1.05 and 1.11 for the ALL and RECENT, respectively. The fact that the number of effective founders is almost the same as effective ancestors is possibly due to a lack of preferential use of some genetic lines by the breeders, avoiding imbalance in founder contribution.

Over half the population is composed of base animals. The RECENT population shows higher number of founders and ancestors than the ALL population. This is due to the rapid growth in the herd in recent years along with a higher number of founder herds because new farmers entered the breeders association. The number of animals in ALL population with both parents known is lower than that of the RECENT population. As can be seen there was a recent increase in animals registered with a single parent known which could have contributed to this. The herd book for males closed in 2009 while for females it remains open.

The effective size of the population and effective number of founders and ancestors were higher than for other breeds found in the literature, indicating a broader genetic base or lack of older pedigree information of the Pantaneiro horse. The number of founders for this breed is considerably higher than found by Valera et al. (2005) on Andalusian horses in Spain or Haflinger in Italy (Sabbioni et al., 2007). This is probably because the pedigree of the Pantaneiro is much shallower than these breeds which are over 100 years old and so much more information on parents is available. The Pantaneiro herd book also accepts animals without parental identification into it.

Although the population here was small, the inbreeding is less than found in the international breeds. This may be due to careful choice of stallions in the conservation herd, guided by the breeders association or EMBRAPA, lack of pedigree information (Sabbioni et al, 2007), few generations with pedigree information, as well as individual breeder preference. Ideal inbreeding rates vary between 0.05 (Nicholas, 1989) and 0.01 (FAO, 1998) per generation. This would indicate that current levels are favorable for the Pantaneiro horse but it should be remembered that registration is on the increase and Welsh et al. (2010) also showed low initial inbreeding for commercial pig breeds in the US, but in later generations it increased to high levels. The predicted curve in the present study (Figure 3) shows that inbreeding cannot be ignored if conservation issues are to be met, especially with closure of the herd book.

The average relatedness has remained relatively stable in the last two generations. This may be due to improved attention to avoid matings between relatives. Average relatedness provides complementary information to values of inbreeding to explain mating relatives (Gutierrez et al., 2003). In conservation genetics, knowledge of relatedness is required to optimize conservation strategies. Coancestry and relatedness are expressed relative to the base population, in which all alleles are defined as not being identical by descent, so that coancestry in the base population is zero by definition (Falconer & Mackay, 1996; Lynch & Walsh, 1998).

There is a large variation for generation intervals in the literature. Some breeds are used as sports horses and breeding only starts after their sporting career has finished, while the Pantaneiro is normally a working horse and breeding starts earlier (about four years of age). Other reasons reported for long generation intervals are linked to inbreeding leading to poorer reproductive performance (Valera et al., 2005).Generation intervals for this breed are significantly lower than found for other breeds (Dias et al., 2000; Vicente et al., 2009; Mota et al., 2006), which was 9.5 to 10.5 years in horses more commonly used for sport or leisure but closer to that for the Campolina (Procópio et al., 2003), which is also a working breed. This may be due to earlier breeding and as this is a working breed animals are replaced by younger, fitter animals at a younger age than horses used for recreational purposes. Generation intervals for this breed are significantly fewer in number than in other breeds, such as 19 for the Haflinger in Italy (Sabbioni et al., 2007), but the number of complete generations was similar (3), also reflecting the short breeding life of the animals here. Using the RECENT population, generation intervals

are longer (Table 3) probably due to the more complete pedigree and increased interest in the use of this horse for sports competitions, further delaying reproduction.

The lower generation intervals for dams than sires is contrary to that found by Sabbioni et al. (2007), who found smaller generation intervals for the paternal line. These are considerably less than those found in Andalusian horses in Spain (Valera et al., 2005), Trakehner in Germany (Teegan et al., 2009) or Haflinger in Italy (Sabbioni et al., 2007). As these are working horses usually dealing with *Bos indicus* cattle in harsh environmental conditions (flooding, high temperatures and humidity), injuries and sickness are frequent which can reduce productive life of these animals.

The increase in inbreeding found, especially in the most recent generation, may be due to a deeper, higher quality pedigree in recent years. The increase in inbreeding was reflected in the N_e, which decreased. This may also be due to the fact that the owners do not register all animals as foals or register animals at an older age, as adult horses may be registered in the breed. Young animals only receive temporary register. If both parents have a definitive register they can be added to the closed herd book when they become adults. If only one parent is known, females can be registered in the definitive register of the open herd book but the herd book for males was closed in 2008.

Moureaux et al. (1996) reported that when inbreeding using pedigree studies on horse breeds are considered, two groups of horse breeds may be distinguished: one group of international breeds, showing values ranging from 0.81% to 2.89%, and the other group with small population sizes with values ranging from 2.25% to 14.7%. In the case of the Pantaneiro, herd numbers are low but so is inbreeding, which is desirable for a conservation breed.

The average relatedness (AR) herein was low compared with other horse breeds (Valera et al., 2005; Zechner et al., 2002). Valera et al. (2005) found that average values of inbreeding and AR for the whole Andalusian horse population were, respectively, 8.48% and 12.25%, while Cervantes et al. (2008) found average inbreeding of 7% for the Spanish Arab. The AR increased up to generation 3 and has remained relatively stable since.

The number of animals in the present generation of the Pantaneiro horse may still increase, but it needs careful attention. In the present study there were on average 2.56 offspring per mare and 12.75 per stallion, which may be considered low, as reproductive technologies such as embryo transfer and artificial insemination are not used in this breed. The number of progeny per stallion for the Haflinger (Sabbioni et al., 2007) was, on average, 22.34 (7.88 males and 14.46 females), and mares produced, on average, 3.91 foals (1.76 males and 2.15 females). This is probably a reflection of the fact that these are working horses that are replaced more frequently than horses used for leisure and is also a reflection of the harsh environmental conditions in which they live.

The N_e quantifies genetic drift and the rate of inbreeding in a population (Teegan et al., 2009). N_e has been used as a key parameter in designing strategies for the definition and conservation of endangered animal species (Bijma et al., 2001). According to the classification given by the Food and Agriculture Organization of the United Nations (FAO, 1998), based on the overall population size, the number of breeding females and the trend in population size, the Pantaneiro horse can be described as endangered-maintained.

There was a great similarity between F_{ST} values for sire and farms, indicating a lack of exchange of sires between farms, although most farms (Table 5) buy in stallions (or have bought in the past). In the studied population F_{IS} values indicate some heterozygosity between farms but not municipalities (it is possible that within municipalities the offspring come from the same few foreign sires), with overall F_{IT} close to zero. These results indicate that future breeding plans should include exchange of sires between municipalities to maintain low inbreeding levels and genetic variation. F_{IT} can be partitioned into F_{ST} because of the Wahlund effect (reduction of heterozygosity in a population caused by subpopulation structure) and F_{IS} because of inbreeding. F_{ST} (which is similar to Nei´s distance) can range from 0 (no genetic differentiation) to 1 (subpopulations fixed for different alleles). F_{IS} can range from −1.0 (all individuals heterozygous or totally outbred) to +1.0 (no observed heterozygotes or totally inbred) (Wright, 1978), while F_{IT} is the mean reduction in heterozygosity of an individual relative to the total population.

Effective number of herds has steadily increased over the generations, which is a measure of success for the Pantaneiro Horse Breeding Program. Alderson (1990) used the GCI to calculate the effective number of founders in the pedigree of an animal. The higher the GCI value the higher the values of an animal for conservation. The GCI can be used either by individual breeders as an aid to the selection of a herd sire or within an overall breed policy to formulate a group breeding program. However, the index has limitations such as not accounting for any concentration of breeding to non-founder animals in subsequent generations in a pedigree and is inapplicable without pedigree records (Alderson, 1992). The GCI indices are considerably lower than those found for the Spanish pure bred (Valera et al., 1998). This is possibly due to lack of pedigree information.

Conclusions

Genetic parameters from genealogical data of the Pantaneiro horse population show that inbreeding is under control. No subpopulation structure was seen in the analysis but use of sires and dams is usually limited within small geographical ranges and long generation intervals with few offspring left per mare/stallion. Future breeding plans should include more germplasm exchange between municipalities. An increase in the effective population size should also be a priority for the breed.

Acknowledgements

References

ABREU, U.G.P.; McMANUS, C.; SANTOS, S.A. Cattle ranching, conservation and transhumance in the Brazilian Pantanal. **Pastoralism – Research, Policy, Practice**, v.1, p.99-114, 2010.

ALDERSON, L. **Genetic conservation of domestic livestock**. Wallingford, U.K.: CAB International, 1990. 242p.

ALDERSON, L. A system to maximize the maintenance of genetic variability in small populations. In: ALDERSON, L.; BODO, I. (Eds.) **Genetic conservation of domestic Livestock II**. Wallingford: CABI, 1992. p.18-29.

BALLOU, J.D.; LACY, R.C. Identifying genetically important individuals for management of genetic diversity in pedigreed populations. In: BALLOU, J.D.; GILPIN, M.; FOOSE, T.J. (Eds) **Population management for survival & recovery. Analytical methods and strategies in small population conservation**. New York: Columbia University Press, 1995. p.76-111.

BIJMA, P.; VAN ARENDONK, J.A.M.; WOOLLIAMS, J.A. Predicting rate of inbreeding for livestock improvement schemes. **Journal of Animal Science**, v.79, p.840-853, 2001.

BOICHARD, D.; MAIGNEL, L.; VERRIER, E. The value of using probabilities of gene origin to measure genetic variability in a population. **Genetics Selection Evolution**, v.29, p.15-23, 1997.

CABALLERO, A.; TORO, M.A. Interrelations between effective population size and other pedigree tools for the management of conserved populations. **Genetic Research Cambridge**, v.75, p.331-343, 2000.

CABALLERO, A.; TORO, M.A. Analysis of genetic diversity for the management of conserved subdivided populations. **Conservation Genetics**, v.3, p.289-292, 2002.

CECCHI, F.; CIAMPOLINI, R.; CIANI, E. et al. Demographic genetics of the endangered Amiata donkey breed. **Italian Journal of Animal Science**, v.5, p.387-391, 2006.

CERVANTES, I.; MOLINA, A.; GOYACHE, F. et al. Population history andgenetic variability in the Spanish Arab Horse assessed via pedigree analysis. **Livestock Science**, v.113, p.24-33, 2008.

DIAS, I.M.G.; BERGMANN, J.A.G.; REZENDE, A.S.C. et al. Formação e estrutura populacional do eqüino brasileiro de hipismo. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.52, n.6, p.647-654, 2000.

DUBOIS, C.; RICARD, A. Efficiency of past selection of the French Sport Horse: Selle Français breed and suggestions for the future **Livestock Science**, v.112, p.161-171, 2007.

FALCONER, D.S.; MacKAY, T.F.C. **Introduction to quantitative genetics**. 4.ed. Edinburgh: Longman Group Limited, 1996. 464p.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO. **Primary guidelines for development of national farm animal genetic resources management plans**. 1998. Available at: <http://lprdad.fao.org/cgi-bin/getblob.cgi?sid5-1,50006090> Accessed on: Feb. 1, 2010.

GOYACHE, F.; GUTIÉRREZ, J.P.; FERNANDEZ, I. et al. Using pedigree information to monitor genetic variability of endangered populations: the Xalda sheep breed of Asturias as an example. **Journal of Animal Breeding Genetics**, v.120, p.95-103, 2003.

GUTIÉRREZ, J.P.; ALTARRIBA, J.; DÍAZ, C. et al. Pedigree analysis of eight Spanish beef cattle breeds. **Genetics Selection Evolution**, v.35, p.43-63, 2003.

GUTIÉRREZ, J.P.; CERVANTES, I.; MOLINA et al. Individual increase in inbreeding allows estimating effective sizes from pedigrees. **Genetics Selection Evolution**, v.40, p.359-378, 2008.

GUTIÉRREZ, J.P.; GOYACHE, F. A note on ENDOG: a computer program for analysing pedigree information. **Journal of Animal Breeding and Genetics**, v.122, p.172-176, 2005.

JAMES, J.W. Computation of genetic contributions from pedigrees. **Theoretical and Applied Genetics**, v.42, p.272-273, 1972.

LACY, R.C. Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. **Zoo Biology**, v.8, p.111-123, 1989.

LYNCH, M.; WALSH, J.B. **Genetics and analysis of quantitative traits**. Sunderland: Sinauer Assocs. Inc., 1998. 980p.

MacCLUER, J.W.; BOYCE, A.J.; DYKE, B. et al. Inbreeding and pedigree structure in Standardbred. **Journal of Heredity**, v.74, p.394-399, 1983.

McMANUS, C.M.; SANTOS S.A.; SILVA, J. et al. Body indices for the Pantaneiro horse. **Brazilian Journal of Veterinary Research and Animal Science**, v.45, p.362-370, 2008.

MEUWISSEN, T.H.E.; LUO, Z. Computing inbreeding coefficients in large populations. **Genetics Selection Evolution**, v.24, p.305-313, 1992, doi:10.1186/1297-9686-24-4-305.

MOTA, M.D.S.; PRADO, R.S.A.; SOBREIRO, J. Characterization of the Mangalarga horse population in Brazil. **Archivos de Zootecnia**, v.55, p.31-37, 2006.

MOUREAUX, S.; VERRIER, É.; RICARD, A. et al. Genetic variability within French race and riding horse breeds from genealogical data and blood marker polymorphisms. **Genetics Selection Evolution**, v.28, p.83-102, 1996.

NICHOLAS, F.W. Incorporation of new reproductive technology in genetic improvement programs. In: HILL, W.G.; MACKAY, T.F.C. (Eds) **Evolution and animal breeding**. Wallinford: CAB International, 1989. p.201-209.

PONCET, P.A.; PFISTER, W.; MUNTWYLER, J. et al. Analysis of pedigree and conformation data to explain genetic variability of the horse breed Franches Montagnes. **Journal of Animal Breeding and Genetics**, v.123, p.114-121, 2006.

PROCÓPIO, A.M.; BERGMANN, J.A.G.; COSTA, M.D. Formação e demografia da raça Campolina. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.55, p.361-365, 2003.

REGE, J.E.O.; GIBSON, J.P. Animal genetic resources and economic development: issues in relation to economic valuation. **Ecological Economics**, v.45, p.319-330, 2003.

SABBIONI, A.; VALENTINO, B.; FRANCESCA, T.M. et al. Genetic variability and population structure in the Italian Haflinger Horse from pedigree analysis. In: ANNALI FACOLTÀ DI MEDICINA VETERINARIA DI PARMA, 2007, Parma. **Annals…** Parma, Italy, 2007. v.27, p.199-210.

SANTOS, A.S.; SERENO, J.R.B.; MAZZA, M.C.M. et al. Origin of the Pantaneiro horse in Brazil. **Archivos de Zootecnia**, v.41, p.371-381, 1992.

SANTOS, S.A.; McMANUS, C.M.; MARIANTE, A.S. et al. **Estratégias de conservação in situ do cavalo Pantaneiro**. Corumbá: EMBRAPA, 2003. 29p.

TEEGAN, C.; EDEL, C.; THALLER, G. Population structure of the Trakehner Horse breed. **Animal**, v.3, p.6-15, 2009.

UNEP-WCMC. **Global list of transboundary protected areas** (2007). Available at:<http://www.tbpa.net/docs/pdfs/2007_UNEPWCMC_Global_List_of_Transboundary_Protected%20Areas.pdf.> Accessed on: July 15, 2011.

VALERA, M.C.; MOLINA, A.A.; RODERO, F.A. Genetic conservation index in the Carthusian strain of the Andalusian horses. **Archivos de Zootecnia**, v.47, p.175-180, 1998.

VALERA, M.; MOLINA, A.; GUTIÉRREZ, J.P. et al. Pedigree analysis in the Andalusian horse: population structure, genetic variability and influence of the Carthusian strain. **Livestock Production Science**, v.95, p.57-66, 2005.

VICENTE, A.; CAROLINO, N.; GAMA, L.T. Demographic parameters in Lusitano Horse. **Archivos de Zootecnia**, v.58 (Suppl. 1), p.501-504, 2009.

WELSH, C.S.; STEWART, T.S.; SCHWAB, C. Pedigree analysis for 5 swine breeds in the United States and the implications for genetic conservation. **Journal of Animal Science**, v.88, p.1610-1618, 2010.

WRIGHT, S. **Evolution and the genetics of populations**. Volume 4: Variability within and among natural populations. Chicago: University of Chicago Press, 1978. 590p.

ZECHNER, P.; SÖLKNER, J.; BODO, I. et al. Analysis of diversity and population structure in the Lipizzan horse breed based on pedigree information. **Livestock Production Science**, v.77, p.137-146, 2002.