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Short Communication

Studies of blood groups and protein polymorphisms in the Brazilian horse breeds Mangalarga Marchador and Mangalarga (*Equus caballus*)

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Abstract

Allelic frequencies at 12 loci (five blood groups: C, D, K, P, and U; and seven protein polymorphisms: Al, A1B, Es, Gc, Hb, PGD, and Tf), are given for two Brazilian horse breeds: Mangalarga Marchador and Mangalarga. The high genetic identity value found (96.0%) is consistent with their common origin, although, at some point of the development of Mangalarga Marchador, Mangalarga separated from the original stock. The expected average heterozygosity was higher in Mangalarga Marchador. The populations presented genetic differentiation, as shown by the statistically significant value of F_{ST}. The nonsignificant F_{IS} values showed that there was no appreciable consanguineous mating in any of the two populations. Exclusion probability calculated for the 12 loci was 87.0% and 86.5% for Mangalarga Marchador and Mangalarga, respectively. No genetic equilibrium was observed in the A1B, Tf, and Es loci of Mangalarga Marchador. The frequencies of blood factors A, Q, and T were calculated.

Key words: blood groups, biochemical polymorphisms, breeds, horses.

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Introduction

Mangalarga Marchador and Mangalarga are two Brazilian-developed equine breeds which were greatly influenced by horses brought from the Iberian Peninsula during the colonization period. Crosses of Alter breed studs with Andalusian and Creole-type mares began in the mid-nineteenth century in the State of Minas Gerais, paving the way for the breed that was to be named Mangalarga Marchador. Interested as they were in a faster horse, breeders in the State of São Paulo crossed these animals with Thoroughbred, Arab, Anglo-Arab and American Saddle Horse breeds, leading to the beginning of Mangalarga. The Mangalarga stud book was opened in 1934 and closed in 1943. For the Mangalarga Marchador breed, it was opened in 1949 and closed in 1979 for stallions, and in 1984 for mares (Bortoni, 1990; Simões, 1979).

The analysis of genetic markers, such as blood groups and biochemical polymorphisms, allows the characterization and evaluation of intra- and interpopulation genetic variability, thus being a useful tool for characterizing and comparing breeds.

The aim of this study was to assess and compare the genetic variability in a sample of Mangalarga Marchador and Mangalarga breeds through the study of eight blood groups and seven biochemical polymorphism systems.

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Material and Methods

A total of 1,360 Mangalarga Marchador (n = 680) and Mangalarga (n = 680) horses were analyzed. All horses had been registered with the breed associations, and were adult males and females from different herds. They had been blood-typed in the laboratory, as part of a pedigree control program sponsored by the breed associations. Two 10mL blood samples were collected from each horse, one with either ACD or heparin as a anticoagulant, as the red cell source, and one in a dry tube, as the serum protein source.

Standard serological tests involving agglutination and complement-mediated lysis (Stormont and Suzuki, 1964) were used to detect red cell allo-antigens at eight blood group loci: A, C, D, K, P, Q, U, and T. Twenty-seven blood factors were tested: Aa, Ab, Ac, Ag; Ca; Da, Db, Dc, Dd, De, Df, Dg, Dh, Di, Dk, Dl, Do; Ka; Pa, Pb, Pd; Qa, Qb, Qc; Ua; and V, W. Except for factors V and W, which belong to the putative T system, all the others are internationally recognized. The reagents used to detect the antigenic specificities were polyclonal monospecific allo-antisera, standardized by international cooperation. Rabbit serum previously absorbed by a pool of horse red cells was used as the complement source.

Standard methods of starch and polyacrylamide gel electrophoresis (Gahne, 1966; Bengtsson and Sandberg,

1973; Juneja *et al.*, 1978) were used to identify variants of the following red cell and serum protein systems: albumin (Al), A1B glycoprotein, esterase (Es), Gc protein, 6-phosphogluconate dehydrogenase (PGD), and transferrin (Tf). Corn starch (Penetrose 30) substituted potato starch. Polyacrylamide gel isoelectric focusing was used to detect hemoglobin (Hb) variants (Ryder *et al.*, 1979).

All seven biochemical polymorphism loci were considered as closed systems, with no silent allele and with each phenotype defining a single genotype. Among the blood groups, only system D was considered as closed, although some alleles were masked by others.

Allelic frequencies for the electrophoretic systems were estimated by simple allele counting. For the blood groups systems, C, K, and U, which present one factor and two alleles each, the allelic estimation was done by the square root method. For the purpose of allelic calculation, considering Pa and Pb factors only, the P system is analogous to the ABO system in humans. Based on this, Bernstein's equation was used (in: Beiguelman, 1981). The method described by Braend (1963), with modifications, was used for the D system, and involved direct counting of recognizable genotypes and allocation of ambiguous phenotypes. Genetic equilibrium was assumed for calculating allelic frequencies of the blood group loci. For the blood group systems A, Q, and T, only the frequency of each antigenic factor was calculated, due to the impossibility of assigning genotypes to the majority of the animals tested. The comparison of these frequencies between breeds was made by a contingency table chi-square.

The Hardy-Weinberg equilibrium was tested for the seven protein polymorphism loci using the chi-square test at a 5 percent significance level. Intrabreed genetic variation was quantified by measuring the average expected heterozygosity (He). The inbreeding coefficient (F_{IS}) and fixation index (F_{ST}) were estimated according to Weir and Cockerham (1984), using the FSTAT software (280 randomizations) (Goudet, 1995). Nei's genetic identity (I) was employed to estimate the similarity between the breeds (Nei, 1972). In order to establish the efficacy of these genetic markers in parentage control, exclusion probability was calculated for the seven biochemical polymorphism loci and for the blood group systems C, D, K, P, and U (Jamieson, 1965).

Results

Allelic frequencies of blood groups and protein polymorphisms are shown in Table 1. Worth noting is the absence of variants $A1B^F$ and Tf^M in both populations, of K^a and D^{dl} in Mangalarga Marchador, and of D^{dekl} and PGD^D in Mangalarga. The comparison between observed and expected genotypes for the seven biochemical polymorphism loci showed that both populations were in equilibrium for part of them, but the Mangalarga Marchador breed presented a significant deviation at loci A1B, Es, and Tf.

Table 1 - Allelic frequencies of blood groups (C, D, K, P, and U) and biochemical polymorphism loci in Mangalarga Marchador (MM) and Mangalarga (ML) breeds.

Systems	Alelles	MM	ML	Systems	Alelles	MM	ML
C	C^a	0.647	0.885	A1B**	$A1B^K$	0.956	0.991
	C^-	0.353	0.115				
					$A1B^S$	0.043	0.009
					$A1B^F$	0.000	0.000
D	D^{adl}	0.216	0.111				
	D^{bc}	0.155	0.256	Tf**	Tf^D	0.630	0.573
	D^{cegi}	0.001	0.001				
	D^{cfgk}	0.017	0.001		Tf^{F1}	0.002	0.024
	D^{cg}	0.039	0.049				
	D^{dekl}	0.006	-		Tf^{F2}	0.166	0.197
	D^{delo}	0.030	0.188				
	D^{del}	0.188	0.058		Tf^H	0.014	0.003
	D^{dfkl}	0.168	0.168				
	D^{dgh}	0.118	0.082		Tf^J	0.004	0.001
	D^{dkl}	0.062	0.081				
	D^{dl}	-	0.006		Tf^M	0.000	0.000
					Tf^O	0.035	0.014
					Tf^R	0.150	0.188
				Hb*	$Hb^A{}_1$	0.004	0.010
					$Hb^A{}_2$	0.100	0.430
					$Hb^B{}_1$	0.581	0.320
					$Hb^B{}_2$	0.315	0.240
K	K^a	0.000	0.005	Es	Es^F	0.331	0.043
	K^-	1.000	0.995				
					Es^I	0.667	0.950
					Es^S	0.002	0.007
U	U^a	0.480	0.591	Gc**	Gc^F	0.965	0.990
	U^-	0.520	0.409				
					Gc^S	0.035	0.009
Al	Al^A	0.479	0.710	PGD	PGD^F	0.956	0.979
	Al^B	0.521	0.290		PGD^D	0.014	0.000
					PGD^S	0.030	0.018

" - ": allele not found in this sample; */**: N = 680, except for breed ML, in which *: 475, and **: 522.

The frequencies of the A, Q, and T system blood factors are presented in Table 2. Aa, Ac, and Ag of system A, and Qa and Qb of system Q showed a significant deviation between breeds.

The components of the F-statistics are shown in Table 3. The mean F_{IS} values for Mangalarga Marchador and Mangalarga (-0.023 and 0.007, respectively) were nonsignificant, whereas the mean F_{ST} value (0.117) was significant.

The expected heterozygosity, exclusion probability, and genetic identity values are shown in Table 4. Mangalarga Marchador showed greater intrapopulation genetic variability (0.36), as compared to the Mangalarga

Table 2 - Blood factor frequencies of systems A, Q, and T in Mangalarga Marchador (MM) and Mangalarga (ML) breeds.

Factors	Aa	Ab	Ac	Ag	Qa	Qb	Qc	V	W
MM	0.67	0.13	0.04	0.10	0.63	0.73	0.74	1.00	0.09
ML	0.90	0.12	0.12	0.19	0.12	0.18	0.80	0.10	0.10

Table 3 - Inbreeding coefficient (F_{IS}) and fixation index (F_{ST}) for the protein systems in Mangalarga Marchador (MM) and Mangalarga (ML) breeds, with their probabilities (P).

System	F_{IS} MM	P	F_{IS} ML	P	F_{ST}	P
Al	0.009	0.432	-0.008	0.579	0.104	0.001
Tf	-0.017	0.768	0.049	0.075	0.004	0.001
Es	-0.089	0.993	0.062	0.075	0.307	0.001
PGD	0.000	0.621	0.126	0.032	0.005	0.023
A1B	0.094	0.036	-0.009	1.000	0.022	0.001
Gc	-0.038	1.000	-0.008	1.000	0.015	0.001
Hb	-0.021	0.746	-0.035	0.850	0.130	0.001
Mean	-0.023	0.946	0.007	0.329	0.117	0.001

Table 4 - Expected heterozygosity, exclusion probability (%), and Nei's genetic identity of Mangalarga Marchador (MM) and Mangalarga (ML) breeds for 12 loci.

System	Expected heterozygosity		Exclusion probability (%)		Nei's Identity
	MM	ML	MM	ML	
C	0.46	0.20	1.01	0.01	0.93
D	0.84	0.84	43.74	44.61	0.79
K	0.00	0.01	0.00	0.43	1.00
P	0.29	0.13	0.16	0.07	1.00
U	0.50	0.48	3.52	1.66	0.97
Al	0.50	0.41	18.80	16.40	0.91
Tf	0.55	0.60	32.40	35.00	0.99
Es	0.45	0.10	17.50	4.60	0.91
PGD	0.08	0.04	4.10	1.70	1.00
A1B	0.08	0.02	3.90	9.00	0.99
Gc	0.07	0.02	3.20	9.00	0.99
Hb	0.55	0.65	28.30	36.90	0.78
Mean	0.36	0.29	86.97	86.52	0.96

breed (0.29). Systems D, Al, Hb, and Tf showed the highest intralocus heterozygosity in both breeds.

Genetic identity estimation revealed that the similarity of these breeds was 96.0%. The exclusion probability calculated based on these 12 loci was 87.0% for Mangalarga Marchador, and 86.5% for Mangalarga. The most informative loci for parentage analysis were those of systems D, Hb and Tf.

Discussion

The genetic disequilibrium observed in the Mangalarga Marchador population could be interpreted as a result of allelic introgression from other breeds, before the registry book had been closed.

The average expected heterozygosities obtained for Mangalarga Marchador and Mangalarga breeds (0.36 and 0.29, respectively) were similar to the values found for domestic breeds, which vary from 0.25 to 0.44 (Bowling, 1994). Cothran *et al.* (1998) found a higher observed heterozygosity in the Mangalarga Marchador than in the Mangalarga breed.

Tf^{J} and D^{cfgk} are considered to be specific alleles of the Andalusian (Spanish Purebred Horse) and related breeds (Kaminski and De Andrés, 1986). The occurrence of these alleles in the animals studied by us evidences the influence of this breed on the formation of Mangalarga Marchador and Mangalarga. The phenogroup D^{dekl} found in the Barb horse (Ouragh *et al*., 1994) was observed only in the Mangalarga Marchador population, showing the influence of the Barb horse on its formation, through the Iberian Peninsula breeds.

The nonsignificant mean F_{IS} values for Mangalarga Marchador and Mangalarga indicate the absence of appreciable consanguineous mating within the populations. The mean F_{ST} (0.117), which was significant, shows that these populations exhibit a genetic differentiation. About 11.7% of this differentiation can be ascribed to genetic differences among them, while about 88.3% is found within the breeds.

Nei's genetic identity (96.0%), calculated for 12 loci, revealed the high similarity of these breeds, although there are genetic differences between them. Systems D and Hb, however, were less similar.

The lower observed frequencies of the Tf^{J} and D^{cfgk} variants and the loss of D^{dekl} and PGD^{D} in Mangalarga support the idea that this breed derived from Mangalarga Marchador, having lost some variability in this process, which suggests a founder effect. This is in accordance with the higher heterozygosity observed in the Mangalarga Marchador breed. Although the absence of the D^{dekl} and PGD^{D} variants in Mangalarga should be confirmed in a larger sample of animals, their frequencies are expected to be low.

The most informative loci for parentage control were D, Hb, and Tf, which account for 72.7% of the total exclusion probability (87.0%) in Mangalarga Marchador, and for 77.3% of the total exclusion probability (86.5%) in Mangalarga.

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