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Vinocur, Myriam Elizabeth; Brass, Karin Erica; Batistella Rubin, Mara Iolanda; Silva Mondino, Carlos Antonio

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Variabilidade genética de cavalos da raça crioula no Brasil

Myriam Elizabeth Vinocur[1] Karin Erica Brass[2] Mara Iolanda Batistella Rubin[3] Carlos Antonio Mondino Silva[3]

ABSTRACT

Allelic frequencies of 7 blood groups and 8 protein systems were determined in 6 herds of Criollo horse breed raised in Rio Grande do Sul, Brazil. Analysis of these frequencies showed a significant isolation component (Fst = 0.0866; p<0.01) and construction of dendogram using Nei´s D confirmed this difference among the 6 herds. The highest values measuring genetic variability on 15 blood types were average heterozygosity: 0.4631; total number of alleles: 87 and probability of exclusion: 98%. When all herds were considered together, the inbreeding level (Fis) was zero. These results indicate that the Criollo horses have a large genetic variability.

Key words: *blood groups, protein systems, geneticvariability horse*.

ABSTRACT

As freqüências alélicas de sete sistemas de grupos sangüíneos e oito sistemas protéicos foram determinadas em seis rebanhos de cavalos Crioulos criados no Rio Grande do Sul, Brasil. A análise destas freqüências indicou que os rebanhos apresentaram um significativo componente devido ao isolamento (Fst = 0,0866; p<0,01) e esta diferença foi confirmada a partir do dendograma construído, utilizando-se a distância de Nei. Na medição da variabilidade genética, utilizando os 15 sistemas de tipagem sangüínea, os valores mais altos encontrados foram heterozigose média: 0.4631; número total de alelos :87 e probabilidade de exclusão de um parentesco indicado: 98%. Quando todas os rebanhos foram considerada na análise, o nível de endocruzamento (Fis) foi zero. Estes resultados indicam que os cavalos Crioulos apresentam ampla variabilidade genética.

Palavras-chave: *grupos sangüíneos, sistemas protéico, variabilidade genética, eqüinos.*

INTRODUCTION

Genetic markers such as blood groups, protein systems and DNA polymorphisms are used as tools to study genetic diversity within and between horse breeds (STORMONT et al., 1963; ELLEGREN et al., 1992; BOWLING, 1997). Criollo horses, in Brazil, descend from Iberian Peninsula horse breeds brought to South America by Portuguese and Spanish settlers. The Stud Book of the Brazilian Criollo Horse Breeder Association (ABCCC) started in 1932 and remained open until 1944. In 1950 the Brazilian, Chilean, Argentinean and Uruguayan Criollo Horse Breeder

[1] Aluna de doutorado do Programa de Pós-gradução em Medicina Veterinária da Universidade Federal de Santa Maria (UFSM).
[2] Médico Veterinário, Doutor, Professor Adjunto, Departamento de Clínica de Grandes Animais, UFSM, Faixa de Camobi, Km 9, 97105-900, Santa Maria, RS. E-mail: kbrass@zaz.com.br Autor para correspondência.
[3] Médico Veterinário, Doutor, Professor Titular, Departamento de Clínica de Grandes Animais, UFSM

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Associations, standardized their pedigree and their registration on the Stud Book record. From that period until now Brazilian horse breeders imported Criollo horses from Uruguay, Argentina and Chile in order to improve genetic value and conformation aspects. They are resistant and used mostly in cattle work and, currently, in the "*Freio de Ouro*" Competition. Mating close relatives was a common practice. In the 90's the annual registration rate of ABCCC was about 9,000 foals per year. In this study, six different herds of Brazilian Criollo horses were used to measure their genetic variability using 7 blood group loci and 8 protein systems. Data generated were used to determine the relationship among herds aiming to integrate them into one large population and/or to study the genetic behavior in two of those herds that bred free of other Brazilian Criollo breeders influence. The allele frequencies were calculated to evaluate parentage exclusion probability for this breed.

MATERIAL AND METHODS

Blood samples from 208 Criollo horses bred in Rio Grande do Sul – Brazil were collected in 1998, following the technique described by BOWLING & CLARK (1985). These horses, chosen at random, were originated from five different farms located in 5 different counties in the state of Rio Grande do Sul as follow: 51 horses from Bagé (BG), 44 from Santana do Livramento (SL), 16 from São Sepé (SS), 22 from Santa Maria (SM) and 23 from Cruz Alta (CA). A 6th group was formed by 52 horses sampled during the "Expointer 98" Horse Show in Porto Alegre (PA), Brazil. This group was taken as a representative sample since they were selected horses from different breeding farms, considered the best of the breed. Breeding selection overall was based mostly on fenotype, where a particular stallion is mated to a variable, but usually high, number of mares. Breeders from BG and SL farms maintain a closed breeding system following a line breeding program, based on breeding stock from Uruguay (BG) and Argentine (SL).

Blood samples were used to determine seven internationally recognised blood group loci: A, C, D, K, P, Q and U by standard immunological procedures involving microhemagglutination and complement mediated hemolysis (LEONG & TERASAKI, 1985) and 8 protein systems: albumin (Al), transferrin (Tf), A1B glycoprotein (Xk), group-specific component (Gc), esterase (Es) protease inhibitor (Pi) hemoglobin (Hb) and 6-phosphogluconate dehidrogenase (6-PGD) by starch, agarose and polyacrylamide gel electrophoresis (SCOTT, 1970; JUNEJA ***et al***., 1978; TROMMERHAUSEN-SMITH & SUZUKI, 1978). Allelic frequencies for C, K and U blood groups were calculated by the square-root method. Frequencies for blood groups A, P and Q were calculated using the allocation method (ANDERSSON, 1985). System D frequencies were determined by direct counting, considering all phenogroups and assuming the possible genotypes of each one. The allelic frequencies of 8 protein loci, whose genotypes could be directly inferred from phenotypes, were calculated using the GENEPOP program, version 1999 (RAYMOND & ROSSET, 1995). The genetic variation measures of observed heterozygosity (Ho), Hardy-Weinberg expected heterozygosity (He), maximum heterozygosity in equilibrium (Hm), number of alleles per locus (k), effective number of alleles (ne) and F-statistic components (Fis = inbreeding level, Fst= genetic differentiation) were calculated only for protein loci because of the presence of recessive alleles and/or ambiguous genotypes at blood group loci. Allelic frequencies at 15 blood type loci were used to estimate probability of exclusion (PE, JAMIESON 1965) and average heterozygosity (H). A phylogenetic tree (dendrogram) was constructed using the unweighted pair group-method with arithmetic mean (UPGMA) and the matrices of standard genetic distances (D) between samples (NEI, 1972).

RESULTS

The allelic frequencies at 7 blood groups (bg) and 8 protein systems (ps) in the 6 herds of Brazilian Criollo horses are indicated in table 1 and 2. The total number of alleles (k) at 15 blood typing loci in each herd were distributed as follows: in the PA sample k = 87 (bg 44, ps 43), in the BG herd k = 81 (bg 45, ps 36), in the CA herd k = 65 (bg 33, ps 32), in the SS farm k = 63 (bg 30, ps 33), in the SM farm k = 59 (bg 26 , ps 33) and in the SL farm k = 72 (bg 38, ps 34). All loci, except the K blood group in the CA herd showed more than one allele. Allelic frequencies were different between herds. The transferrin variant J related with Iberian Peninsula-derived breeds appeared in a low frequency in the BG herd. Table 3 shows the measures of the genetic variability using 8 protein loci. Assuming the Hardy-Weinberg equilibrium, the Al and Pi systems in the SL herd and the Tf system in the SS herd showed deviation when the expected and the observed heterozygosity (Ho) were compared with maximum heterozygosity in equilibrium (Hm). The number of effective alleles (ne) in each system explains the higher occurrence and significance of common alleles over the less frequent ones. Inbreeding coefficient (Fis) of the SS herd differed

Table 1 – Allelic frequencies of 8 serum protein loci in 6 Criollo horse herds. Mn = modal number.

		Herds					
Systems	Alleles	PA	BG	CA	SS	SM	SL
		(Mn = 52)	(Mn = 51)	(Mn = 23)	(Mn = 16)	(Mn = 22)	(Mn = 44)
Hb	A	0.020	-	0.021	0.094	-	0.023
	AII	0.039	0.143	0.042	0.156	0.114	0.023
	B	0.422	0.163	0.313	0.500	0.364	0.727
	BII	0.520	0.694	0.625	0.250	0.523	0.227
6-PGD	F	0.953	0.898	0.935	0.846	0.864	1.000
	S	0.047	0.102	0.065	0.154	0.136	0.000
Al	A	0.337	0.294	0.500	0.531	0.341	0.670
	B	0.663	0.706	0.500	0.469	0.659	0.330
XK	F	-	0.010	-	-	-	-
	K	0.885	0.618	0.935	0.875	0.886	0.977
	S	0.115	0.373	0.065	0.125	0.114	0.023
Gc	F	0.933	0.961	0.978	0.906	0.932	0.955
	S	0.067	0.039	0.022	0.094	0.068	0.045
Es	F	0.154	0.049	0.109	0.031	0.045	0.284
	G	0.087	0.108	0.239	0.063	0.045	0.057
	H	0.010	-	-	0.000	0.000	0.023
	I	0.702	0.608	0.630	0.875	0.841	0.636
	M	0.019	-	0.000	-	0.068	-
	R	0.010	0.010	0.022	-	-	-
	S	0.019	0.225	-	0.031	-	-
Tf	A	0.020	0.186	0.022	0.031	0.045	-
	D	0.176	0.520	0.348	0.094	0.273	0.068
	D_2	0.020	-	-	-	-	-
	E	0.020	-	-	-	-	-
	F_1	0.216	0.020	0.087	0.156	0.159	0.057
	F_2	0.284	0.069	0.217	0.188	0.295	0.568
	F_3	0.010	-	-	-	0.023	0.023
	H_1	0.010	0.010	-	0.031	-	0.034
	H_2	0.020	0.088	-	-	0.023	-
	J	-	0.020	-	-	-	-
	O	0.206	0.078	0.217	0.438	0.136	0.227
	R	0.020	0.010	0.109	0.063	0.045	0.023
Pi	G	0.040	-	0.045	0.031	-	-
	I	0.020	0.078	0.045	0.031	0.091	0.102
	K	-	0.020	-	0.031	-	-
	L	0.420	0.343	0.318	0.313	0.114	0.170
	L_2	0.040	0.010	-	0.188	0.023	0.034
	N	0.100	0.078	0.273	0.125	-	0.114
	O	0.020	-	0.023	0.094	-	0.023
	P	0.020	-	-	-	0.023	0.011
	Q	-	-	-	0.031	-	-
	R	0.100	0.225	0.068	0.125	0.091	0.057
	S	0.040	0.069	0.068	0.031	0.136	0.205
	T	0.060	0.020	0.068	-	0.045	0.091
	U	0.060	0.137	0.045	-	0.227	0.125
	V	-	-	0.023	-	-	0.011
	Z	0.060	-	0.023	-	0.023	-
	Others	0.020	0.020	-	-	0.227	0.057

from zero in the Pi system. The same occurred in the PA sample and in the SL herd in the Tf system. Average heterozygosity (H) in the BG and SS herds was higher than in the PA, CA and SM herds. The lowest average heterozygosity was observed in the horses from the herd located in SL. H and standard error (se) estimated using 15 blood typing systems was 0.4945 (se: 0.0673), 0.4945 (se: 0.0673), 0.4784 (se: 0.0743), 0.4887 (se: 0.0626), 0.466 (se: 0.0662) and 0.4566 (se: 0.0698) for the PA, BG, CA, SS, SM and SL herds, respectively.

Table 3 - Measurement of genetic variation in 6 Criollo horse herds at 8 biochemical polymorphic systems. H(o), H(e), H(m) observed, expected and maximum heterozigosity in equilibrium, respectively; ne= effective number of alleles; Fis=population inbreeding level; H=average heterozygosity. Mn = modal number.

Systems	Gene variation	*Herds* PA (Mn = 52)	BG (Mn = 51)	CA (Mn = 23)	SS (Mn = 16)	SM (Mn = 22)	SL (Mn = 44)
Hb	Ho	0.667	0.551	0.500	0.562	0.591	0.318
	He	0.556	0.476	0.520	0.675	0.595	0.423
	Hm	0.750	0.667	0.750	0.750	0.667	0.750
	ne	2.25	1.91	2.08	3.08	2.47	1.73
	Fis	-.202	-.159	+.040	+.172	+.007	+.250
6-PGD	Ho	0.094	0.204	0.130	0.308	0.273	-
	He	0.091	0.185	0.125	0.271	0.241	-
	Hm	0.500	0.500	0.500	0.500	0.500	-
	ne	1.10	1.23	1.14	1.37	1.32	-
	Fis	-0.040	-.103	-.048	-.143	-.135	-
Al	Ho	0.481	0.353	0.478	0.562	0.500	0.523
	He	0.451	0.419	0.511	0.514	0.460	0.447
	Hm	0.500	0.500	0.500	0.500	0.500	0.500
	ne	1.82	1.72	2.04	2.06	1.85	1.81
	Fis	-.067	+.160	+.066	-.098	-.090	-.172
XK	Ho	0.192	0.372	0.130	0.250	0.227	0.045
	He	0.206	0.484	0.125	0.226	0.206	0.045
	Hm	0.500	0.667	0.500	0.500	0.500	0.500
	ne	1.26	1.34	1.14	1.29	1.26	1.05
	Fis	+.068	+.233	-.048	-.111	-.105	-.012
Gc	Ho	0.135	0.078	0.043	0.187	0.136	0.091
	He	0.127	0.076	0.043	0.175	0.130	0.088
	Hm	0.500	0.500	0.500	0.500	0.500	0.500
	ne	1.14	1.08	1.04	1.21	1.15	1.10
	Fis	-.063	-.031	-	-.071	-.050	-.036
Es	Ho	0.558	0.529	0.652	0.250	0.182	0.545
	He	0.480	0.571	0.545	0.236	0.291	0.516
	Hm	0.857	0.800	0.750	0.750	0.750	0.750
	ne	1.92	2.33	2.20	1.31	1.41	2.07
	Fis	-.164	+.074	-.202	-.062	-.097	-.057
Tf	Ho	0.725	0.588	0.826	0.875	0.818	0.477
	He	0.805	0.682	0.782	0.758	0.808	0.622
	Hm	0.909	0.889	0.833	0.857	0.875	0.857
	ne	5.12	3.15	4.58	4.13	5.20	2.65
	Fis	+.100*	+.139	-.058	-.160	-.013	+.235 *
Pi	Ho	0.720	0.804	0.909	0.625	0.954	0.954
	He	0.802	0.802	0.821	0.849	0.865	0.883
	Hm	0.923	0.900	0.909	0.900	0.900	0.917
	ne	5.06	5.05	5.60	6.61	7.40	8.58
	Fis	+.105	-.002	-.110	+.270*	-.107	-.081
H		**0.4396**	**0.4622**	**0.4340**	**0.4631**	**0.4490**	**0.3783**

* significant at the 5% level.

Hierarchic structure of the localities

The inbreeding coefficient (Fis) and the effects of subpopulation subdivision (Fst) are indicated in Table 4. When each and all loci were considered together Fis did not differ from zero. The Fst was high for the Hb, Al, Xk, Es, Tf, and Pi systems ($p<0.01$). The dendrogram of 6 Brazilian Criollo horse herds constructed by Nei's distance (Nei´s D) using 15 blood typing systems and 100 bootstrap replications is shown in Fig. 1. PA and SM herds were the closest related; SL and BG herds were the most distant related. The probability of exclusion for detecting incorrectly assigned parentage using 15 blood typing systems in each studied Criollo herds from Rio Grande do Sul – Brazil is shown in Table 5. The effectiveness of these tests was over 95% for all 6 herds.

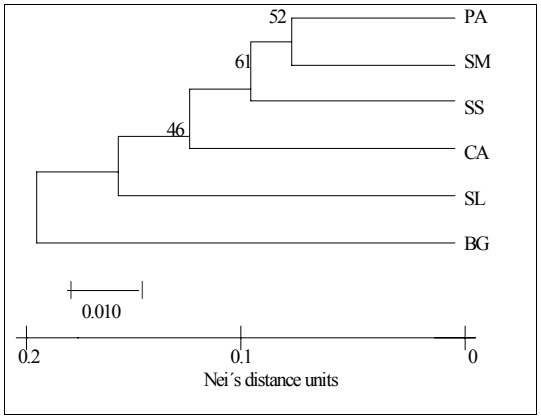

Figure1 – Phylogenetic tree (dendrogram) of 6 Criollo horse herds calculated using Nei´s modified genetic distance and 100 bootstrap replications.

DISCUSSION

This report shows the genetic variability of 6 Brazilian Criollo herds at 15 blood typing systems. The 6 herds showed a moderate degree of differentiation as expressed by their allele frequencies (Table 1 and 2) and subpopulation subdivision effect (Fst). Results indicated the analysis of the 6 herds as one large population (Table 4) is incorrect. SL and BG herds were not close related to the other herds, as expected and demonstrated by Nei's distance (NEI, 1972), probably because they kept a closed breeding program (Figure 1). Although the number of allele variant in BG and PA herds was similar (k=81 and k= 87, respectively). The wide genetic variability present in the Criollo breed is due to the contribution of each herd. The inbreeding coefficient (Fis) was not significant (Table 4). Looking at Fis for each locus in each sample (Table 3) it was observed that the most polymorphic systems Tf and Pi in the PA, SL and SS herds showed significant levels of inbreeding. The high polymorphism of these systems indicated a trend in gene frequency. The force by which allelic and genotypic frequencies change in a wild population could be natural selection, gene flow from one population to another, random drift and deviation from random mating (FUTUYMA, 1996). A small population size, a low gene flow and the use of nonrandom breeding practices, based mainly on phenotype and performance results, may have influenced the isolation effect and the inbreeding level found in the 6 Brazilian Criollo herds.

Brazilian Criollo horses are Iberian Peninsula-derived breeds as well as Criollo horses from Argentina (ARC) and Chile (CHC) and other breeds from South America such as Mangalarga (M), Campolina (C) Mangalarga Marchador (MM), Brazilian Pantanal horses (BP). Due to the lack of information about the genetic variability of these domestic breeds, the comparison was only possible measuring the average of heterozigosity. The average of heterozigosity in the 6 herds of Brazilian Criollo horses was: PA: 0.4396, BG: 0.4622, CA: 0.4340, SC: 0.4631, SL: 0.3783 and in other breeds it was: ARC: 0.410, CHC: 0.428, M: 0.328, C: 0.410, MM:0.411, BP: 0.387. (COTHRAN ***et al***., 1993; PERAL-GARCIA ***et al***., 1996; COTHRAN ***et al***., 1998; HAU ***et al***., 2000). High values of heterozigosity average could be expected to correlate with adaptive responses to environmental challenges (HEDRICK ***et***

Table 4 - Components of F- statistics for 8 biochemical polymorphic systems including 6 Criollo horse herds from Rio Grande do Sul, Brazil.

Systems	Fis	X_2	Fst	X_2	d.f
Hb	-0.0226	0.1052	0.1380	170.568**	15
6-PGD	-0.1000	2.0200	0.0315	12.726	5
Al	-0.0262	0.1428	0.0918	38.189**	5
XK	0.1143	2.7305	0.1444	120.141**	10
Gc	-0.0491	0.5038	-0.0051	2.122	5
Es	-0.0650	0.8830	0.0564	140.774**	30
Tf	0.0810	1.3581	0.1089	495.931**	55
Pi	-0.0100	0.0180	0.0393	212.220**	75
Total	0.0015	0.0037	0.0866	11271,856**	200

**significant at the 1% level.
d.f .- Degrees of freedom

Table 5 - Probability of exclusion (PE) using 15 blood typing systems in 6 Criollo horse herds from Rio Grande do Sul – Brazil.

	Herds					
Systems	PA	BG	CA	SS	SM	SL
Blood groups						
A	0.211	0.426	0.266	0.151	0.132	0.224
C	0.124	0.114	0.109	0.122	0.124	0.109
D	0.611	0.643	0.645	0.564	0.499	0.462
K	0.008	0.053	0.000	0.016	0.023	0.086
P	0.204	0.252	0.110	0.081	0.023	0.113
Q	0.295	0.374	0.226	0.215	0.297	0.351
U	0.092	0.104	0.109	0.073	0.056	0.033
PE total	0.864	0.908	0.857	0.786	0.759	0.811
Biochemical polymorphisms						
Hb	0.153	0.111	0.132	0.236	0.169	0.089
6-PGD	0.004	0.017	0.007	0.034	0.028	0.000
Al	0.100	0.086	0.125	0.124	0.101	0.098
XK	0.021	0.115	0.007	0.024	0.020	0.001
Gc	0.008	0.003	0.001	0.015	0.008	0.004
Es	0.123	0.172	0.147	0.027	0.042	0.133
Tf	0.427	0.285	0.370	0.344	0.414	0.220
Pi	0.451	0.435	0.455	0.485	0.527	0.593
PE Total	0.797	0.764	0.781	0.796	0.813	0.775
PE using 15 systems	0.972	0.983	0.969	0.956	0.955	0.957

al., 1986). Brazilian Criollo horses have been submitted to environmental adaptation for centuries, therefore, care should be taken to keep this genetic variability through planned breeding.

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REFERENCES

ANDERSSON, L. The estimation of blood group gene frequencies: A note on the allocation method. **Anim Blood Groups Biochem Genet,**. v.16, p.1-7, 1985.

BOWLING, A.T. **Horse genetics**. New York : CAB International, 1997. 200p

BOWLING, A.T.; CLARK, R.S. Blood group and protein polymorphism gene frequencies for seven breeds of horses in the United States. **Anim Blood Groups Biochem Genet**, v.16, p.93-108, 1985.

COTHRAN, G. et al. Análisis genético del caballo chilote de la isla de Chiloé. **Chile Arch Med Vet**, v.25, p.137-146, 1993.

COTHRAN, G. et al. Genetics of the pantaneiro horse of the Pantanal region of Brazil. **Genetics and Molecular Biology**, v. 21, p.343-349, 1998.

ELLEGREN, H. et al. Cloning of highly polymorphic microsatelites in the horse. **Animal Genetics**, v. 23, p.133-142, 1992.

FUTUYMA, D.J. **Biologia evolutiva**. 4.ed. São Paulo : Triângulo, 1996. 646p.

HAU, J.E.; MIÑANO, H.S.; TUPAC-YUPANQUI, B.I. Genetic analysis in two populations of Peruvian Horse: Peruvian Paso and Blood Horse Race. In: INTERNATIONAL CONFERENCE ON ANIMAL GENETICS, 27., 2000, Minnesota. **Proceedings...** Minnesota : ISAG, 2000. p.20.

HEDRICK, P. W. et al. Protein variation, fitness and captive propagation. **Zoo Biology**, v.5, p 91-99, 1986.

JAMIESON, A.A. The genetics of transferrins in cattle. **Heredity**, v.20, p.419-441, 1965.

JUNEJA, R.K.; GHANE, B.; SANDBERG, K. Genetic polymorphism of the vitamin D binding protein and another post-albumin protein in horse serum. **Anim Blood Groups Biochem Genet**, v. 9, p.29-36, 1978.

LEONG, S.W.; TERASAKI, P.I. Microtest for red cell typing. **Transfusion**, v.25, p.149-151, 1985.

NEI, M. Genetic distances between populations. **Am Nat,** v.106, p.283-292, 1972.

PERAL-GARCIA, P.; KIENAST, M.; VILLEGAS, C.E.E. Estudio de relaciones genéticas entre razas equinas mediante el análisis multivariado. **Agro Sur,** v.4, p.39-47, 1996.

RAYMOND, M.; ROUSSET, F. Population genetics software for exact test and ecumenicism. Genepop (version 1.2). **J Heredity,** v.86, p.248-49, 1995.

SCOTT, A.M. A single acid gel for separation of albumins and trasferrins in horses. **Anim Blood Groups Biochem Genet,** v.1, p.253-254, 1970.

STORMONT, C.J.; SUZUKI, Y.; RHODE, E.A. Serology of horse blood groups. **Cornell Veterinarian,** v.54, p.439-452, 1963.

TROMMERHAUSEN-SMITH, A.; SUZUKI, Y. Identity of XK and Pa systems in equine serum. **Anim Blood Groups Biochem Genet**. v.9, p.127-128, 1978.