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Polymorphism of serum proteins in Campolina horses

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Abstract

To characterize genetically the Campolina horse breed, 218 animals from different herds located in many Brazilian states were analyzed. For this study the serum proteins Albumin, Gc (vitamin D binding protein), Transferrin, Esterase and Xk (pre-albumin) were tested using starch gel electrophoresis and polyacrylamide gel electrophoresis (PAGE). The results indicated high number of homozygous animals in the Campolina breed, probably due to the intense use of some famous stallions.

Keywords: Campolina horses, serum proteins, polymorphism.

Introduction

Considering the equine breeding process, several breed registries have been interested in the study of the genetic structure of the breeds, looking for a better comprehension of the meaning of the genetic variability between individuals or populations. The study of the protein polymorphism became more useful after the development of the electrophoresis procedures, facilitating its detection. The protein variants occur by changes of amino acids or deletions that modify the molecular structure, being characters of simple Mendelian inheritance and codominants. Such knowledge has importance in the studies of breed origin and formation, as well as complement to the parentage tests, increasing their reliability, and also as potential genetic markers for some characteristics of economic interest. From the native breeds, there is few information about their genetic structure. About the Campolina breed, according to Procópio (2000), the herd was formed by the Andalusian and Berbere breeds, with a later introduction of the Mangalarga Marchador breed, which was also formed by horses with a high portion of Andalusian blood (through the Alter breed, from Portugal) and also Berbere blood (Andrade, 2000). Other study conducted by Laat (2001) showed that the current gene pool of the breed derives only from 20 founder animals. This situation, according to the same author, can be negative from the point of view of genetic improvement, since the genetic gain becomes reduced considering the long generation intervals. Based in this study, a program helping to define the direction to be followed by the Brazilian Campolina Horse Breeders Registry (ABCCC), aiming the refinement of the breed and the prevention of the inbreeding consequences was started. Its first goal was to identify the polymorphism of the serum proteins Albumin, Gc (vitamin D binding protein), Transferrina, Esterase and Xk (pre-albumin), including the estimation of the gene and genotype frequencies.

Material and Methods

Serum samples of 218 horses of the Campolina breed, obtained from several herds located in different Brazilian states were analyzed. Part of the samples was collected during the National Exhibition of 2002, occurred at the Exhibition Park of Gameleira – Belo Horizonte, MG, with the cooperation of the Brazilian Campolina Horse Breeders Registry (ABCCC). The other part was collected from different herds by an authorized technician of ABCCC.

The samples were collected in sterile vacuum tubes, without anticoagulant, in a volume of 5,0ml. Afterwards, the serum was separated in aliquots of 1,0ml and divided into lots of 24 samples.

The electrophoresis technique in starch gel was used for the identification of Albumin and Transferrin alleles (according to Scott, 1970) and of Esterase alleles (according to Braend, 1973). For the systems Gc, Transferrin, Esterase and Xk the PAGE (polyacrylamide gel electrophoresis) technique described by Juneja *et al*. (1978) was used. It must be emphasized that for Albumin, Esterase and Transferrin the PAGE gel is used to confirm some alleles identified in the starch gels, but some other alleles can only be visualized in one of them, starch or polyacrylamide. The gene and genotype frequencies were estimated using the software Computerization of Chi-square for Hardy-Weinberg Equilibrium[3].

Results

For Albumin, a high frequency of the allele **B** (0,77) was observed in the sampling, as well as the frequency of the genotype **BB** (0,60), according to the results showed in Tables 1 and 2, respectively.

[3] Software developed by Bernoco, D. (1997) used under license.

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Table 1. Albumin, transferrin, esterase, Gc and Xk gene frequency in the Campolina breed

System	Allele	N° of alleles	Gene frequency.
Albumin	A	98	0,2240
	B	338	0,7750
Transferrin	O	132	0,3020
	D	158	0,3620
	R	24	0,0550
	F_1	18	0,0410
	F_2	102	0,2330
	H_2	2	0,0040
Esterase	I	390	0,6900
	F	85	0,2010
	G	48	0,1070
Gc	F	404	0,9300
	S	52	0,0730
Xk	K	390	0,8940
	S	31	0,0710
	F	15	0,0340

Table 2. Albumin transferrin, esterase, Gc and Xk genotype frequency in the Campolina breed

System	Genotype	N° of individuals observed/genotype	Genotype frequency
Albumin	AA	08	0,0360
	AB	78	0,3570
	BB	132	0,6050
Transferrin	RO	04	0,0180
	DO	54	0,2470
	F_1O	07	0,0320
	F_2O	32	0,1460
	DF_1	05	0,0220
	DF_2	44	0,2010
	F_2R	05	0,0220
	DH_2	01	0,0045
	F_2H_2	01	0,0045
	DR	10	0,0457
	OO	14	0,0642
	F_1F_1	03	0,0137
	F_2F_2	12	0,0550
	DD	21	0,0963
	RR	04	0,0183
	F_1R	01	0,0045
Esterase	II	102	0,4670
	FI	52	0,2380
	FF	18	0,0820
	GG	01	0,0040
	GI	42	0,1920
Gc	FF	186	0,8500
	FS	32	0,1460
Xk	KK	177	0,8110
	KS	31	0,0710
	FK	10	0,0450

In the tests for Transferrin, the alleles **O, D, R, F_1, F_2** and **H_2** were observed. The alleles **O** and **D** were the most frequent, followed by the allele **F_2** and finally **H_2** allele. The values observed for the gene frequencies are shown in Table 1 and the Table 2 shows the genotype frequencies observed in the system.

For Esterase the alleles **I**, **F** and **G** were observed. The gene and genotype frequencies are shown

For the protein Gc in the analyzed sample, both lleles world-wide well-known in the system, **F** and **S** were detected. The gene and genotype frequencies are shown in Tables 1 and 2.

For the Xk the alleles, **K**, **F**, and **S** were observed in the sampling, being the gene and genotype frequencies shown in Tables 1 and 2, respectively.

Discussion

The present study reflects the results obtained by Procópio (2000), who reported inbreeding higher than 6%, with increase of 1,9% per generation. This inbreeding becomes more evident mainly when the frequencies of some genotypes are observed, such as: Albumin-BB (0,6050), Esterase-II (0,4670), Gc-FF (0,8500) and Xk- KK (0,8110). These values are an indicative of a large number of homozygous individuals in the breed. Still explaining such findings, it should be considered the reports of Laat (2001) showing that, from the current gene pool in the breed, 50% of the genes came from less than 20 animals, being also observed that some important stallions for the breed remained in use for long a time. This author analyzed data of 15667 animals, considering births occurring from the year of 1951 to 2000.

Other fact that can reinforce the affirmative of high inbreeding in the Campolina breed is the confirmation, after pedigree analysis of all the animals, that 40% of them were sons or grandsons of the stallions *Desacato da Maravilha* and *O.P. de Santa Rita*. In many cases these stallions appeared in both sides of the genealogy. Moreover, when considering the genotypes of these stallions for the analyzed proteins, the results[4] were: for *Desacato da Maravilha* – **Tf** – F_2O, **Es** – FI, **Xk** – KK, **Gc** – FF and for *O.P. de Santa Rita* **– Tf –** DO**, Es –** II**, Xk –** KK**, Gc –** FF. Such findings can also help to explain the high frequencies observed for some alleles and, in the case of the allele F of the protein Gc, the value of 0,9300 indicates that this allele is almost fixed in the breed.

This can also help to understand the findings of Fonseca (1973) who reported, as probably caused by inbreeding depression, a small percentage of live foals in mates involving close related animals. This author found, for these animals, a large number of abortions and stillborn records, citing as possible causes, the quality and/or quantity of gametes produced by them, as well as alterations in sexual behavior and quality of the uterine environment in females.

Conclusions

The obtained data can be useful in future works that aim to deepen the knowledge on the genetic origin of the Campolina breed. Moreover, the results could also supply the Brazilian Campolina Horse Breeders Registry (ABCCC) with information for inbreeding control programs, that according to previous reports, already brought undesirable consequences to the breed.

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[4] It was not possible to determine the genotype of the stallions for the protein Albumin.