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Possible association between hemoglobin types and reproductive disorders in Brazilian Mangalarga mares*

Possível associação entre tipos de hemoglobina e problemas reprodutivos em éguas Mangalarga brasileiras

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SUMMARY

In the present report the biochemical polymorphism of Mangalarga mares hemoglobin, in reproductive age, from Santa Fé Farm, Botucatu, São Paulo, was studied. Animals were classified in two groups, according to reproductive history of each mare; the first group was performed by normal mares (control group) and the second one by animals with reproductive disorders (barren mares). From each animal, around 15 ml of vessel blood were collected. Hemoglobins were typed by polyacrylamide gel electrophoresis, 7% of concentration in the resolving gel, in a discontinuous alkaline (pH 8.6) buffer system. The following hemoglobins phenotypes were found in the control group, with the respective frequencies: A_1---(2.0%), $A_1A_2m^+m^+$(21.0%) and $A_1A_2m^+m^-$(27.0%). To the group performed by reproductive disorders carrier animals the following results were obtained A_1---(10.0%), $A_1A_2m^+m^+$(12.0%) and $A_1A_2m^+m^-$(28.0%). The difference observed in the A_1--- phenotype between the groups may be due to a probable liaison with hemoglobin locus and another one related with reproductive traits. Besides this fact, tropical environment effects may be acting on this locus, thus leading to obtained results.

UNITERMS: Hemoglobin; Electrophoresis; Mares.

INTRODUCTION

Mangalarga Horse breed has as main former the Andalusian. In the beginning of this century other breeds were introduced, like Arab, Anglo-Arab (Arab x Thoroughbred), Thoroughbred and American Saddle Horse, according to Simões[20] (1978). In São Paulo it is possible to note three lineages originated in the south of Minas Gerais. Presently, the Mangalarga breed is mainly based in four breeds. Although this equine breed has been diffused into almost all the Brazilian country, hence becoming a national breed and no more a regional breed, a high uniformity degree among the animals is noted. An important cause of infertility or subfertility, especially in older, multiparous mares is the condition of persistent endometritis, which represents an economic impact on the horse-breeding industry. Endometritis can be defined as an infection or inflammation of the endometrium which may not be discernible on rectal examination, as stated by Threlfall[22] (1980). It is perpetuated by anatomic, physiologic and immunologic failures in mares, as stated by Asbury[1] (1983). Most of the bacteria associated with uterine disease are opportunistic pathogens normally found on the external genitalia of clinically normal mares and stallions. Probably, they are transported to uterus during coitus. Most mares are resistant to intrauterine infection and rapidly eliminate bacteria without treatment, while others apparently are unable to eliminate bacteria quickly, and so develop a chronic endometritis, according to Hughes; Loy[14] (1975). This increasing susceptibility to bacterial colonization of the uterus seems to be more common in older mares, but it is caused by an individual variation, as affirmed by Blue *et al.*[3] (1982). Blood proteins show a polymorphism due to genetical variance. Such variants are transmitted by simple Mendelian heritage, with no dominance. To explain the hemoglobin bands intensity variation in horses, the existence of a locus that would control its synthesis is admitted. Generally, the variants are detected by electrophoretic methods because aminoacids change and/or deletions modify molecules structure and/or charges. The first genetical variation in horses hemoglobin was described by Cabannes; Serain[8] (1955). Bangham; Lehmann[2] (1958), employing paper electrophoresis, found two fractions in 64 animals and one horse showing only the fast component, confirming data

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obtained by Cabannes; Serain[8] (1955). Meanwhile, the same authors used starch gel electrophoresis and observed that the same animal had two hemoglobin fractions, in a ratio of 0.25:1, which led the authors to conclude that there would be more than one locus controlling hemoglobins expression. Braend; Stormont[6] (1964) observed that 183 horses' blood samples showed the same type of hemoglobins; two components, the fast one moved with the same mobility of the B bovine fraction and the slow was intermediary between A and B bovine fractions. Kilmartin; Clegg[15] (1967) investigated by chromatography the aminoacid sequence in the equine hemoglobin types. They observed that the difference in electrophoretic mobilities was due to aminoacid changes at position 60 in α chain. Glutamine, a neutral aminoacid, appeared in the fast component, while it was changed by lysine in the slow one, which shows positive charge in an alkaline pH. This fact would explain the difference in mobility. Braend[4] (1967) analyzed horse hemoglobin samples by electrophoresis and quantified components A_1 and A_2. In horses from various races three phenotypes appeared, as previously described by Braend; Efremov[5] (1964). One of them showed only the fast component (A_1---). The others showed two bands (A_1A_2), but with quantitative differences among them. Component A_2 was slower in alkaline pH. A group had around 38% of A_2, that varied from 34% to 41%; another class showed around 18% of A_2 varying from 15 to 23%. With the aim of explaining such variations, authors admitted that another locus would control horse hemoglobin synthesis. This locus was named as modulator locus Hb^m, which would influence the slow hemoglobin component (A_2) by the inhibition of a structural gene. This gene would be responsible for the synthesis of one polypeptidic chain from Hb A_2. If there is homozygosity in the locus ($Hb^m Hb^m$), and in such a way that a complete inhibition occurs, the slow component will not appear. Only 20% of the slow component will appear if only one of the alleles is active ($Hb^{m+}Hb^{m+}$). The present work was done with the aim of studying the genetical polymorphism of hemoglobins in blood samples from normal mares and from mares Mangalarga breed with reproductive disorders. A probable association between phenotypes and reproductive disorders was also investigated and studied.

MATERIAL AND METHOD

In the present report, 100 Mangalarga mares, from one farm located in Botucatu (Santa Fé), São Paulo, Brazil, aging from 4 to 27 years, with different numbers of birth supplied by the Brazilian Association of Mangalarga Horses breeders (ABCCM). Animals were classified in two groups, one constituted by 50 healthy considered mares, through reproductive history and clinical general examination (G_1). They were considered able to mating and conceiving, and gestation diagnose was confirmed by rectal evaluation and by consecutive ultrasonography (scanners 450 VET, Piemedical, Netherlands); the other group (G_2) constituted by 50 mares with reproductive disorders, which were bred at least in two cycles during the same breeding season, but did not conceive. Such animals were submitted to clinical examination and to cytological and microbiological test to determine the ethiology of the disorder. From each animal 15 ml of blood sample were obtained by venobleeding. Erythrocytes were obtained by three consecutive isosmotic saline washing. Hemoglobin solution was obtained by hiposmotic cell rupture as stated by Ramos[18] (1986), and was kept under refrigeration (-20°C) until use. Hemoglobins were submitted to alkaline vertical polyacrylamide gel electrophoresis, in a discontinuous buffer system, 7% of concentration in the resolving gel according with Davis[11] (1964). Slabs were stained with Amido Black (0.5% w/v in acetic acid 7% v/v) and submitted to densitometry (Zeiss MD 100). Mares were classified according to electrophoretic patterns described by Braend[4] (1967). Descriptive statistic was employed as recommended by Zar[24] (1984). Groups were compared by means of X^2, in basis of Hardy-Weinberg, as recommended by Crow; Kimura[10] (1970). Possible associations were analyzed by Variance Analysis as described by Brow; Forsyte[7] (1974), and Tuckey test.

RESULTS

Hemoglobins showed patterns of one or two bands and the slow one presented differences in staining intensity, as may be seen in Fig. 1. Phenotypes' frequencies are described in Tab. 1 showing that only 4% of healthy mares had phenotype A_1---, while 20% of problem animals showed the same

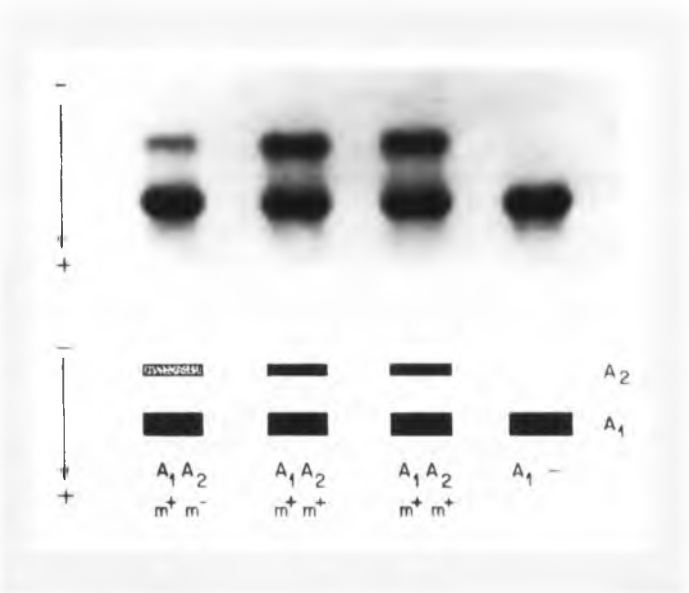

Figure 1
Electropherogram showing obtained results in alkaline vertical slab polyacrilamide gel electrophoresis 7%, pH 8.6, phenotypes A_1---, $A_1A_2m^+m^+$ and $A_1A_2m^+m$.

phenotype. Tab. 2 shows there were differences (X_2) between the considered groups. According to information from ABCCM relative to number of births until 1992 and ages of mares, in the group performed by normal animals ages varied from 4 to 27 years, and in the unhealthy mares from 4 to 25 years. Tab. 3 describes the number of colts by mare in the following age classes: 4 to 7 years, 8 to 14 years and 15 to 27 years.

DISCUSSION AND CONCLUSIONS

Results relative to electrophoretical fractioning of hemoglobins were in agreement with Cabanes; Serain[8] (1955); Bangham; Lehmann[2] (1958); Braend; Efremov[5] (1964); Braend; Stormont[6] (1964); Braend[4] (1967); Sandberg; Bengtsson[19] (1972) and Ezcurra; Mitat[12] (1973). Hemoglobins were named according to nomenclature proposed by Braend[4] (1967), which considers the action of modulatior genes on synthesis of the slow component α chain. Clegg[9] (1970) proposed another nomenclature, employing aminoacid sequence, determining the ones which occupied positions 24 and 60. However, such determination was not possible to obtain. Obtained results showed similar patterns to the published by Lucas; Becari[16] (1986) and Singhvi; Khanna[21] (1987). Analyzing the hemoglobin locus all the population as a single group too, it was observed that the population is under Hardy-Weinberg equilibrium. It wouldn't have to occur, because in the beginning of the breed formation there was a certain inbreeding, as stated in Simões[20] (1978). This fact drives to the conclusion that elected characteristics to the improvement of this race had no sufficient influence on the hemoglobin locus equilibrium. When the population was divided in two groups according to reproductive traits, data displayed in Tab. 2 show that there are differences between healthy an unhealthy mares. Such differences occur due to the high frequency of A_1--- phenotype in the unhealthy group, and to the $A_1A_2m^+m^+$ phenotype in the group of healthy mares, according to Tab. 1. There are two hypotheses to explain such high frequencies. The first one could be a linkage between Hb locus (a genetical marker) and another, at least related to some reproduction characteristics. The second, heterosis, where heterozigote mares to the A locus ($A_1A_2m^+m^-$) could show a positive effect that would improve reproductive traits. These hypotheses may be enforced by results obtained by Velhankar *et al.*[23] (1977), who observed that hemoglobin heterozygote of Gyr heifers became able to reproduction earlier than homozygote ones. In contrast, homozygote lambs to hemoglobin showed better reproductive index than heterozygote according to Hanrahan *et al.*[13] (1978) and Milewski[17] (1983). This controversy may be explained by the fact that Gyr and Mangalarga mares, studied in the present report, were developed under tropical conditions. Environment, then, brought some influence over genome, selecting a more adequate phenotype group. The same may be said about lambs which showed controversial data when compared with the ones presented here. Those animals formed a group developed and reared different environmental conditions, which exercised different selective forces and led to different phenotypic hemoglobins frequencies.

Table 1
Horse hemoglobin patterns phenotypic frequencies in reproductively healthy mares (G_1) and in unhealthy mares (G_2) of Mangalarga breed. Botucatu - SP, 1991/1992.

Groups	Phenotypes	Number	Phenotypic frequencies
G_1	A_1---	10	0.20
	$A_1A_2m^+m^+$	20	0.40
	$A_1A_2m^+m^-$	28	0.56
G_2	A_1---	10	0.20
	$A_1A_2m^+m^+$	12	0.24
	$A_1A_2m^+m^-$	28	0.56

Table 2
Observed and expected distributions of hemoglobins phenotypes in reproductive normal mares (G_1) and barren mares (G_2). Botucatu - SP, 1991/1992.

	G_1			G_2		
	Phenotypes			Phenotypes		
	A_1---	$A_1A_2m^+m^+$	$A_1A_2m^+m^-$	A_1---	$A_1A_2m^+m^+$	$A_1A_2m^+m$
OBS.	2.0	20.0	28.0	10.0	12.0	28.0
EXP.	6.0	16.5	27.5	6.0	16.5	27.5

Table 3
Number of colts by mare distribution under three age classes in reproductive normal mares (G_1) and barren mares (G_2), in Mangalarga breed. Botucatu - SP, 1991/1992.

Age classes	Number of mares	Number of colts	Number of mares	Number of colts
I (4-7 years)	33	36	15	12
II (8-14 years)	15	75	27	98
III (15-27 years)	02	31	08	72

RESUMO

Foram estudadas as hemoglobinas de 100 éguas da raça Mangalarga, em idade de reprodução, provenientes da Fazenda Santa Fé, situada na região de Botucatu. Esses animais foram divididos em 2 grupos, de acordo com o histórico reprodutivo de cada animal, sendo um formado por éguas reprodutivamente normais e o segundo por éguas portadoras de problemas reprodutivos. Foram colhidas amostras de 15 ml de sangue com anticoagulante. As hemoglobinas foram identificadas por meio de eletroforese em gel de poliacrilamida em placa vertical, a 7% em pH 8.6, segundo Davis[11] (1964). Quanto ao sistema de hemoglobinas, foram encontrados os seguintes fenótipos para o grupo de éguas reprodutivamente normais: A_1--- (2,0%), $A_1A_2m^+m^+$ (21,0%) e $A_1A_2m^+m^-$ (27,0%); para o grupo de éguas com problemas reprodutivos: A_1--- (10,0%), $A_1A_2m^+m^+$ (12,0%) e $A_1A_2m^+m^-$ (28,0%). A diferença na freqüência do fenótipo A_1--- entre os grupos pode ter ocorrido devido à existência da ligação do loco hemoglobina a outro que controlaria características de produção. Além disso, pode estar ocorrendo influência do tipo de clima existente nos trópicos.

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