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Study of the mutation causing type 1 polysaccharide storage myopathy in a Mangalarga Marchador population used in breeding programs

Estudo da mutação causadora da miopatia por acúmulo de polissacarídeo tipo 1 em uma população de cavalos Mangalarga Marchador utilizados em programas de melhoramento

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ABSTRACT: The Mangalarga Marchador (MM) breed, which originated in Brazil, constitutes the largest number of horses in the country. The animals are versatile and used in several sports because of major investments made for the genetic improvement of the breed. In recent decades, advances in molecular techniques enabled the identification of genetic diseases in horses. Conducting molecular tests and determining the occurrence of mutations are fundamental for the early identification and prevention of abnormalities. Among the known genetic diseases that occur in horses, the c.926G>A mutation in the *GYS1* gene that causes type 1 polysaccharide storage myopathy (PSSM1) stands out, because it has been identified in several breeds of horses. Although myopathy is common in MM horses, the occurrence of the c.926G>A mutation in the *GYS1* gene has not yet been evaluated. The lack of knowledge about the possible presence of PSSM1 averts the adoption of control measures to prevent the spread of the disease in MM horses. Therefore, the aim of this study was to verify the occurrence of the mutation that causes PSSM1 in MM horses used in breeding programs. Blood DNA was extracted and the region of the *GYS1* gene containing the mutation was amplified and sequenced. No mutation in the *GYS1* gene was found in the evaluated samples. However, since clinical signs of myopathy are frequently observed in MM horses, further studies, including histological analysis, are necessary to establish the underlying causes. In addition, if there is a genetic pattern of occurrence, molecular studies should be considered.

KEYWORDS: PSSM1; myositis; rhabdomyolysis; equine; glycogen.

RESUMO: A raça Mangalarga Marchador (MM), originária do Brasil, constitui a raça de maior número de equinos no país. Os animais são versáteis e utilizados em diversos esportes devido aos seus grandes investimentos em melhoramento genético. Nas últimas décadas, o avanço das técnicas moleculares permitiu a identificação de doenças genéticas em cavalos. A realização de testes moleculares e a determinação da ocorrência de mutações são fundamentais para a identificação precoce e prevenção de anormalidades. Dentre as doenças genéticas conhecidas em equinos, destaca-se a mutação c.926G>A no gene *GYS1* causadora da miopatia por acúmulo de polissacarídeo tipo 1 (PSSM1), pois foi identificada em diversas raças equinas. Embora a miopatia seja comum em cavalos MM, a ocorrência da mutação c.926G>A no gene *GYS1* ainda não foi avaliada. A falta de conhecimento sobre a possível presença de PSSM1 inviabiliza a adoção de medidas de controle para prevenir a disseminação da doença em equinos MM. Portanto, o objetivo deste estudo foi verificar a ocorrência da mutação causadora de PSSM1 em cavalos MM utilizados em programas de melhoramento. O DNA sanguíneo foi extraído e a região do gene *GYS1* contendo a mutação foi amplificada e sequenciada. Nenhuma mutação no gene *GYS1* foi encontrada nas amostras avaliadas. No entanto, como sinais clínicos de miopatia são frequentemente observados em cavalos com MM, mais estudos, incluindo análises histológicas, são necessários para estabelecer as causas subjacentes. Além disso, se houver um padrão genético de ocorrência, estudos moleculares devem ser considerados.

PALAVRAS-CHAVE: PSSM1; miosite; rabdomiólise; equino; glicogênio.

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INTRODUCTION

The Mangalarga Marchador (MM) breed occupies a prominent place in the Brazilian horse breeding, accounting for approximately 11% of the country's equines (ABCMM, 2020; COSTA et al., 2004; INSTITUTO, 2018; LIMA; CINTRA, 2016). During the creation and expansion of MM horses, a broad genetic foundation was employed, with the majority of animals exhibiting an undefined breed and unknown pedigree. These factors contributed to a wide effective population size (BECK, 1992; SANTOS et al., 2019). Initially, the animals were selected for work, but nowadays the selection targets the morphology and performance in gait tests. These selection changes have caused a marked reduction in the effective population size in the last generations of MM horses (BECK, 1992). Genetic diseases usually occur due to high degrees of inbreeding within the same species. Inbreeding aims to standardize, i.e., increase the level of homozygosity, which leads to the production of genetically similar individuals. However, exaggerated inbreeding can increase the chances of undesirable deleterious genes (COSTA et al., 2005; GONÇALVES et al., 2011).

Advances in molecular techniques have enabled the identification of at least 13 genetic diseases in horses, making molecular testing and determination of the occurrence of these mutations fundamental to identify and prevent abnormalities early (ALEMAN, 2008; FINNO; SPIER; VALBERG, 2009; VALBERG, 2018). One of these molecular techniques is PCR (Polymerase Chain Reaction) which uses a set of primers (specific oligonucleotides) to direct the amplification of DNA. The primers are designed to bind to specific regions of the gene of interest. Then, the PCR reaction mixture containing the target DNA, primers, and enzymes is subjected to cycles of heating and cooling that allow the DNA to be amplified in large quantities that can be used to identify genetic mutations (SAIKI et al., 1988).

Type 1 polysaccharide storage myopathy (PSSM1) is an autosomal dominant disease that occurs due to the point mutation c.926G>A in the *GYS1* gene. This mutation results in the replacement of an arginine residue with a histidine residue (R309H), increasing the activity of the enzyme glycogen synthase (GS) and leading to excessive glycogen accumulation in muscle cells (MAILE et al., 2017; MCCUE et al., 2008b) with variable intensity of clinical signs (BROSNAHAN; BROOKS; ANTCZAK, 2010). Previous studies have employed PCR diagnostic technique, to identify PSSM1 in multiple horse breeds (MACCUE et al., 2010; MCGOWAN C.M.; MCGOWAN T.W.; PATTERSON-KANE, 2009; VALBERG, 2018).

Although MM horses consist of a large number of registered animals and have several stud farms dedicated to their breeding in Brazil, there are no studies that have evaluated the presence of PSSM1. This aim of this study was to determine the occurrence of the *GYS1* gene mutation, that causes PSSM1 in MM horses used in commercial breeding programs. The justification is based on the numerous cases of horses presenting clinical signs of myopathy and the need to identify and differentiate the primary causes, for better clinical management of treated animals, as well as to guide breeding programs to prevent the mutation propagation.

MATERIAL AND METHODS

This study was approved by the Ethics Committee on the Use of Animals at the Federal University of Uberlândia, protocol CEUA 089/2018.

Blood samples were collected from the jugular vein of 49 horses used in commercial breeding programs and registered with the Brazilian Association of MM Horse Breeders. The samples were collected from 29 stud farms distributed in 19 cities in the states of Minas Gerais, Goiás, Bahia, Mato Grosso, and Distrito Federal. Stallions (n = 20) and mares (n = 29) had a mean age of 7.5 ± 3.7 and 8.4 ± 5.6 years, respectively.

DNA was extracted from the blood samples using the Illustra™ Blood GenomicPrep Mini Spin Kit (GE Healthcare), according to the protocol recommended by the manufacturer. The purified DNA samples were evaluated for purity (A260/280) and concentration using the NanoDrop® 2000 spectrophotometer (ThermoScientific™) and immediately stored in a freezer at –20°C.

Genotyping of the c.926G>A mutation was performed as described in the literature (DELFIOL et al., 2018). Polymerase chain reaction (PCR) procedures were standardized to amplify the *GYS1* gene region containing the mutation (MCCUE et al., 2008a) using genomic DNA samples and previously designed primer oligonucleotides: forward 5′-GATATCGTGACCCCCAATGG-3′ and reverse 5′-CAGCTGTCCCCTCCCTTAGAC-3′. The reactions were standardized to a final volume of 25 µL, containing 2.5 µL of DNA, 12.5 µL of the enzyme GoTaq® Green Master Mix, 2x (Promega™), 0.6 µL of forward primer, 0.6 µL of reverse primer, and 8.8 µL of nuclease-free water. The thermal cycling conditions were: 95°C for five min, followed by 40 cycles of 95°C for 30 s, 59°C for one min and 72°C for one min, and a final extension cycle of 72°C for five min.

The amplified products were checked by electrophoresis on a 1.5% agarose gel stained with GelRed™ (Biotium, Halward, CA, USA). The gels were photo-documented on ImageQuant® imager (GE Healthcare). The reactions that generated products with a single band and correct size of the amplified fragment were frozen for PCR product purification.

The amplified samples were purified using magnetic beads. Subsequently, they were sequenced and the genotypes of each individual were analyzed for the identification of the target gene in order to determine the occurrence of PSSM1 in the samples.

RESULTS

All 49 samples evaluated were wild type (figure 1), i.e., the c.926G>A mutation in the *GYS1* that causes PSSM1 was not identified in the animals tested.

DISCUSSION

PSSM1 is considered to be the main cause of myopathy in horses (MCCUE et al., 2008b; TRYON et al., 2009). The disease has already been identified in more than 30 breeds of horses, and it is predominantly found in the traction breeds and the Quarter Horse (QH) (MCCOY et al., 2014; MCCUE et al., 2010; MCCUE et al., 2008b; VALBERG, 2018). According to a study carried out in the USA, the highest prevalence of PSSM1 is in the Percheron breed with 62.4% (MCCUE et al., 2010), whereas in QH breed it varied between 11.3% (TRYON et al., 2009) and 6.6%. In Brazil, PSSM1 was evaluated in QH, with a prevalence of 6.7% (DELFIOL et al., 2018). In the clinical routine, it is common to care athletic horses, such as MM, with clinical signs of myopathy which in most cases is of unknown origin or attributed to excessive physical effort without making a differential diagnosis for other causes (VALBERG, 2018).

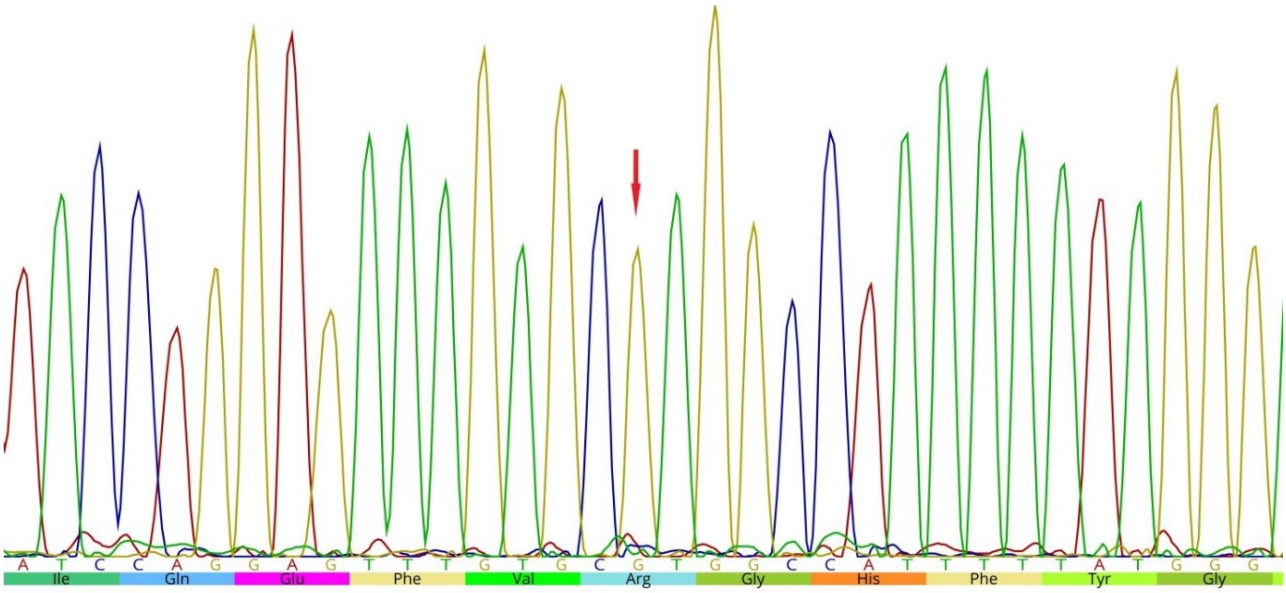
In this study, the identification of the mutation that causes PSSM1 in MM horses was not observed. Studies indicate that the R309H mutation occurs before the separation of domestic horse breeds (MCCOY et al., 2014; MCCUE et al., 2008a), which justifies the investigation of the mutation in MM horses especially on these horses with recurrent myopathy confirmed after excluding other possibilities. Early identification and monitoring of genetic diseases make it possible to arrange mating and prevent the transmission of genetic diseases. It minimizes the financial losses and emotional damage to breeders, in addition to contributing to animal welfare (DELFIOL et al., 2018; VALBERG, 2018).

As reported by some authors (GONÇALVES et al., 2011; SANTOS et al., 2019), increase in consanguinity and reduction of the effective population size of horses, are factors that contribute to the appearance of genetic diseases, a situation observed in breeds such as QH and Percheron that showed a high prevalence of PSSM1 and less genetic diversity (PETERSEN et al., 2013) when compared to MM breed. Although the sample was not large, the horses selected are being used in commercial breeding programs and are considered highlights of MM, having a high potential to disseminate copies of their genes. This suggests that if the mutation was not identified in the sample, it is unlikely to be found in the population.

In MM horses with myopathy without a definite cause, muscle biopsies for histopathological examination could be a useful diagnostic approach; although more invasive, biopsies are important to characterize myopathies in horses. In the USA, these evaluations were a valuable tool to group and characterize muscle diseases in horses (FIRSHMAN; BAIRD; VALBERG, 2005; MCCUE et al., 2008a; VALBERG, 2018).

CONCLUSIONS

The mutation in the *GYS1* gene that causes PSSM1 was not present in the MM horses tested. However, since clinical signs of myopathy are frequently observed in MM horses, further studies, including histological analysis, are necessary to establish the underlying causes. In addition, if there is a genetic pattern of occurrence, molecular studies should be considered.



Fonte: acervo do autor.

Figure 1. Partial chromatogram of wild type (G/G) result (arrow) of the c.926G>A mutation in the *GYS1* gene that causes PSSM1 in the horse Mangalarga Marchador. Image obtained using the Geneious® 10.0 software (Biomatters Ltd, Auckland, New Zealand).

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