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The variant *CLCN1*_c.1775A>C was not Identified in Brazilian Quarter Horses

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ABSTRACT

***Background*:** Congenital myotonia is a genetic neuromuscular disorder characterized by delayed relaxation of the musculature following a strong contraction. Variants in the *skeletal muscle chloride channel 1 gene* (*CLCN1*) have been linked to this disorder across several species. The *CLCN1*_c.1775A>C, an autosomal recessive variant, was identified as a potential causative factor for congenital myotonia in New Forest Pony. While the *CLCN1*_c.1775A>C variant has been studied in different breeds of horses, it remains unexplored in Brazilian Quarter Horses. Therefore, this study aimed to assess the prevalence of the *CLCN1*_c.1775A>C variant among Brazilian Quarter Horses across various disciplines.
***Materials, Methods & Results*:** In this study, 96 DNA samples were obtained from athletic Brazilian Quarter Horses representing various disciplines, 24 each from cutting, reining, barrel racing, and bull-cacthing ("vaquejada"). DNA viability was assessed via PCR targeting the β-*actin* gene. Subsequently, a previously described set of specific primers was employed to amplify the region encompassing the *CLCN1*_c.1775A>C variant. The resulting purified PCR products underwent Sanger direct sequencing, and their electropherograms were analyzed. Notably, none of the horses in this cohort were found to carry the *CLCN1*_c.1775A>C variant. The inbreeding coefficient (*F*) was calculated using pedigree data sourced from the 96 Quarter Horses according to Brazilian Quarter Horse Breeders Association records encompassing 4 generations. The average *F* value for the entire cohort was found to be 0.2%. However, when assessed across disciplines, the average *F* values varied, with cutting at 0.002 (0.2%), reining at 0.003 (0.3%), barrel racing at 0.0008 (0.08%), and bull-cacthing at 0.001 (0.1%), respectively. Notably, within this cohort, 48 horses were identified as inbred, exhibiting an average *F* of 1.5%.
***Discussion*:** Genetic variants associated with conditions such as hyperkalemic periodic paralysis, myosin heavy chain myopathy, and polysaccharide storage myopathy type 1 have been previously documented in Quarter Horses globally, including Brazil. However, as in the present study, the *CLCN1* c.1775A>C variant was also not detected in American Quarter Horses affected by muscular disorders. Although this variant has been implicated as the cause of congenital myotonia in a New Forest pony, its correlation with cases of congenital myotonia in Quarter Horses has not been established yet. Although the inbreeding coefficient and the prevalence of endogamous horses observed in this study were lower compared to findings in other studies, the presence of inbreeding and shared ancestors within Quarter Horses lineages was evident. High rates of inbreeding may disseminate undesirable genetic variants, since popular stallions may improve the athletic performance of its progenies but also may transmit alleles with pathogenic variants, as seen in other genetic disorders in horses. Differently, since it was not observed in this group of evaluated Quarter Horses nor in other previous studies, it may be that the *CLCN1* c.1775A>C is a 'de novo' variant related strictly to congenital myotonia in the New Forest pony. Nevertheless, it is imperative to highlight the potential for congenital myotonia to inflict significant harm upon horses. Investigations into new cases are essential to establish both clinical and etiological diagnoses, thereby enabling the assessment of the requisite preventive measures against this disorder.

Keywords: *CLCN1*, equestrian industry, genotyping, variants.

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INTRODUCTION

Horses play a economic and social role in Brazilian agribusiness, and the Quarter Horses being among the most prominent breeds. This is attributed to their inherent strength, aptitude for learning, agility, speed, large population, and versatility, all of which are showcased across various equestrian disciplines [1,16]. The selective breeding for desirable traits and performance abilities might inadvertently contribute to an increase in the prevalence and frequency of hereditary disorders [26].

Congenital myotonia is characterized by a delay in skeletal muscle relaxing after a vigorous contraction, leading to pain and muscular stifness and its diagnosis is made by genotyping the variant associated with the disorder [27]. Described in goats [6], dogs [8,21,23], buffaloes [7], cats [9,14], sheep [18], pigs [5] and horse [27], congenital myotonia was associated to variants in the *skeletal muscle chloride channel 1* gene (*CLCN1*). The *CLCN1*_c.1775A>C variant, exhibiting a recessive mode of inheritance, was regarded as a potential causative factor for congenital myotonia within the New Forest Pony family. This variant induces a substitution from aspartic acid to alanine at codon 592 (p.D592A) within the well conserved C-terminal cytoplasmic domain of the *CLCN1* gene [27].

While the *CLCN1*_c.1775A>C variant has been studied in different breeds of horses globally [4,27], it remains unexplored in Brazilian Quarter Horses. Therefore, this study aimed to assess the prevalence of the *CLCN1*_c.1775A>C variant among Brazilian Quarter Horses across various disciplines.

MATERIALS AND METHODS

Sampling

The sample size calculation was conducted using OpenEpi software (version 3.0.1)[1] considering the population of 550,000 Quarter Horses registered in the Brazilian Quarter Horse Breeders Association (ABQM) [1], a 5.6% estimated prevalence [27], and a 5% margin of error.

A total of 96 DNA samples obtained from athletic Brazilian Quarter Horses, used in previous studies [2,10,11], representing various disciplines, 24 each from cutting, reining, barrel racing, and bull-cacthing ("vaquejada"). The DNA viability of the samples was assessed via PCR targeting the β-*actin* gene [19]. The sampling was performed under a strict confidentiality agreement to ensure the anonymity of establishments, owners and animals.

PCR technique and sample sequencing

PCR amplification was conducted using previously described set of specific primers [27] targeting the region encompassing the *CLCN1*_c.1775A>C variant. The PCR reaction (total volume: 25 μL) was standardized containing 2.5 μL of template DNA, 12.5 μL of GoTaq® Green PCR Master Mix[2], 8.8 μL of nuclease-free water, and 0.6 μL (300 μM) of each primer. The amplification conditions were as follows: 95ºC for 10 min, followed by 40 cycles of 95ºC for 30 s, 60ºC for 1 min, 72ºC for 1 min, and a final extension at 72ºC for 10 min. Amplicons were analyzed via 1.5% agarose gel electrophoresis and documented with ImageQuant™[3].

The PCR-amplified products were purified utilizing magnetic beads and subsequently subjected to direct Sanger sequencing. The obtained sequences and corresponding electropherograms were analyzed using Geneious® 10.0.9[4], and aligned with the equine *CLCN1* gene sequence available in Genbank™ (XM_001915636).

Data analysis

The inbreeding coefficient (*F*) was calculated using pedigree information from the 96 Quarter Horses (cutting, n = 24 animals; reining, n = 24 animals; barrel racing, n = 24 animals; and "vaquejada", n = 24 animals; n = 59 females and n = 38 males). The pedigree data was collected according to ABQM records with an ancestry of 4 generations. The inbreed average was estimated for all the animals (n = 96 animals) assessed, and from each discipline analyzed, using the software Pedigraph® (2.4 version)[5].

RESULTS

Considering a confidence interval of 95%, the minimum needed sample size, regardless of the assessed variant, was determined to be 82 horses. At the time of sampling, the 96 Quarter Horses included in the present study were confirmed to be healthy. All 96 Quarter Horses assessed were classified as wild-type for the variant *CLCN1*_c.1775A>C.

The inbreeding coefficient of the 96 Quarter Horses analyzed was 0.2% (smallest nonzero: 0.0002; maximum: 0.25), where the average *F* from each dis-

cipline was 0.002 (0.2%) for cutting, 0.003 (0.3%) for reining, 0.0008 (0.08%) for barrel racing, and 0.001 (0.1%) for "vaquejada". Among these 96 horses, inbred was observed in 48 horses of them (average $F = 1.5\%$).

DISCUSSION

Myotonia is a clinical sign characterized by a delay in muscle relaxation after voluntary motor activity or mechanical electric stimulation [7]. The recessive autosomal variant *CLCN1*_c.1775A>C has been exclusively reported in a New Forest Pony with congenital myotonia up to this point [27]. Similar to the current study assessing Brazilian Quarter Horses, the pathogenic allele was not detected in the American Quarter Horses affected by muscular disorders [4]. Despite the absence of the variant CLCN1_c.1775A>C in Quarter Horses, histopathological and electromyographic abnormalities have been documented in Quarter Horses displaying other causes of myotonia or complex repetitive discharges [15,25].

Due to clinical manifestations of pain and muscular stiffness, the congenital myotonia may be mistaken for other common muscle disorders, such as hyperkalemic periodic paralysis (HyPP) [24], malignant hyperthermia (MH) [3], polysaccharide storage myopathy type 1 (PSSM1) [17], and myosin heavy chain myopathy (MYHM) [13], which are presented in Brazilian Quarter Horses [2,10,11].

The *F* is defined as the probability of one individual presenting 2 identical alleles from ancestry [6]. From the 96 Quarter Horses assessed in the present study, the *F* observed was 0.2%, which is lower than those observed in studies associated with the breed (1.07%) and in different modalities (halter, cutting, and racing) [12]. Although the inbreeding coefficient [12,20] and the prevalence of endogamous horses observed in this study were lower compared to findings in other studies [12,22], the presence of inbreeding and shared ancestors within Quarter Horses lineages was evident. High rates of inbreeding may disseminate undesirable genetic variants, since popular stallions may improve the athletic performance of its progenies but also may transmit alleles with pathogenic variants, as seen in other genetic disorders in horses.

Differently, since it was not observed in this group of evaluated Quarter Horses nor in other previous studies [4,27], it may be that the *CLCN1*_c.1775A>C is a 'de novo' variant related strictly to congenital myotonia in the New Forest pony [27]. 'De novo' variants identified in the *CLCN1* gene have been implicated as responsible for causing congenital myotonia in familial groups of animals in other species [5,21,23].

CONCLUSION

Nevertheless, it is imperative to highlight the potential for myotonia congenita to inflict significant harm upon horses. Investigations into new cases are essential to establish both clinical and etiological diagnoses, thereby enabling the assessment of the requisite preventive measures against this disorder.

MANUFACTURERS

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***Declaration of interest*.** The authors declare no conflicts of interest. Only the authors are responsible for the content and writing of this article.

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