Acta Scientiae Veterinariae, 2024. 52: 1963.

RESEARCH ARTICLE
Pub. 1963

ISSN 1679-9216

Degenerative Myelopathy in Brazilian Golden Retriever: Evaluation of the Association with Allele Frequency of the *SOD1*:c.118G>A Variant

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ABSTRACT

***Background*:** Canine degenerative myelopathy represents a progressive neurodegenerative disorder impacting the spinal cord. Initial clinical signs of canine degenerative myelopathy are typically observed in animals around 8 years of age. The clinical signs are characterized by general proprioceptive ataxia and spastic paresis of the pelvic limbs, with an irrepressible progression to flaccid paraplegia after 1 year of the appearance of the signs. Therefore, canine degenerative myelopathy is considered a differential diagnosis for medullary neoplasms, disc protrusion, and hip dysplasia, and since the clinical signs presented by the animals are similar in all these diseases, genetic testing plays an important role in diagnosis. In several dog breeds degenerative myelopathy was linked to the c.118G>A autosomal recessive variant in exon 2 of the superoxide dismutase 1 (SOD1) gene (*SOD1*:c.118G>A). In addition, the *SOD1*:c.118G>A variant in more than 124 breed dogs, suggests that this variant is widely spread and fixed in the species before the breeds' origin. However, there are few studies evaluating this variant in brazilian dogs, and none of them have evaluated it in the Brazilian Golden Retriever dogs. Therefore, this study aimed to evaluate the allele frequency of the *SOD1*:c.118G>A variant in Golden Retriever dogs from Brazilian kennels.

***Materials, Methods & Results*:** A total of 122 Brazilian Golden Retriever DNA samples were used in a PCR procedures with specific primers, previously described, to amplify a 779 base pairs amplicon containing the *SOD1*:c.118G>A variant in this study, and a non-template control reaction was performed to check possible contamination in the PCR preparation. The genotyping of the animals was performed by direct Sanger sequencing of PCR products that amplified a fragment containing the variant. Considering the 95% confidence interval, the sample size used in the present study was slightly larger than the minimum required to assess the allele frequency of *SOD1*:c.118G>A variant (122 versus 113 samples). All animals assessed in this study were clinically normal at the time of sampling and were identified as wild-type for the *SOD1*:c.118G>A variant. Therefore, no alleles of the pathogenic variant (A) were found in the studied population.

***Discussion*:** Although all animals assessed in this study were classified as wild-type, this study is the 1st report in Brazil to evaluate the prevalence of this variant in Golden Retriever dogs. The poor prognosis and inexorable progression of canine degenerative myelopathy force most owners to opt for early euthanasia of affected animals. Knowledge of the disease by owners and breeders, as well as the genotyping of their animals, may prevent the spread of the variant among lineages and also assist veterinarians in differential diagnosis and management of cases of canine degenerative myelopathy. The genetics of Brazilian Golden Retriever were mainly derived from USA bloodlines. With this in mind, we may speculate that the results observed in this study may be influenced by the lower prevalence of the *SOD1*:c.118G>A variant previously found in American Golden Retrievers. The results obtained in the present study, combined with previous studies that described the occurrence of this variant as rare in Golden Retrievers, lead us to speculate that this variant does not impact the Brazilian Golden Retriever dogs.

Keywords: dogs, canine degenerative myelopathy (CDM), neurodegenerative disease, genotyping, *SOD1*, variants.

DOI: 10.22456/1679-9216.140400

Received: 27 May 2024 Accepted: 20 September 2024 Published: 11 October 2024

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INTRODUCTION

Canine degenerative myelopathy (CDM) is a progressive neurodegenerative disease [16] that affects the spinal cord, and its initial clinical signs are observed in animals around eight years old [7]. The clinical signs are characterized by general proprioceptive ataxia and spastic paresis of the pelvic limbs [3,7,18], with an irrepressible progression to flaccid paraplegia after one year of the appearance of the signs [3,7,9,13,18].

The variant c.118G>A in superoxide dismutase1 (SOD1) gene, responsible for CDM in several dog breeds, is considered an autosomal recessive variant with incomplete penetrance [4,13,18], characterized by the replacement of a guanine (G) by an adenine (A) in exon 2 of the *SOD1* gene on chromosome 31 [4]. This variant has been described in several breeds, with its prevalence varying among them [18]. Despite that, few studies used the Golden Retriever breed in their sampling, and the prevalence found in studies ranged from zero [5,6] to 0.03 [18]. Another variant was described in Bernese Mountain dogs; this 2nd variant (*SOD1*:c.52A>T) has been identified as responsible for CDM, exclusively, in this breed [17].

In Brazil, there are 3 case reports published about CDM and the *SOD1*:c.118G>A variant [1,2,14], and only a study analyzing the prevalence of this variant in German Shepherd dogs in Brazil [15]. Because of the huge emotional and financial impact of CDM on the multi-species family, and the important role of the *SOD1*:c.118G>A variant, this study aims to evaluate the prevalence of the variant, in Brazilian Golden Retriever dogs.

MATERIALS AND METHODS

Sample selection

The sampling calculation was performed using OpenEpi software (version 3.0.1)[1]; given the estimated population of 41,000 Golden Retriever in Brazil, the mean allele prevalence of the *SOD1*:c.118G>A variant in several dog breeds of the 8.0% [10,15] with a 5% margin of error.

A total of 122 Golden Retriever DNA samples (67 females and 55 males) were used in this study. These samples were obtained from dog owners and are part of a genetic database belonging to the Laboratory of Molecular Biology of the Veterinary Clinic (LBMCV), and were used in a previous study [16].

PCR and genotype analysis

Primers used in this study were manufactured according to those described by Awano and collaborators [4] (TGGGAGGAGAGTGGAGAACA and TGAGGATTTTCAATGTTTAGGAGTAGC) to amplify an amplicon of 779 base pairs containing the variant.

PCR was performed to a final volume of 20 µL, containing 2.5 µL of template DNA, 10 µL of the enzyme GoTaq® Green Master Mix[2], 5.5 µL of nuclease-free water, and 1 µL of each primer forward and reverse. In addition, a non-template control reaction was performed to check possible contamination in the PCR preparation.

The thermocycling[3] conditions were as follows: 95ºC for 5 min, followed by 35 cycles of 95ºC for 45 s, 60ºC for 35 s, 72ºC for 1 min, and a final extension of 72ºC for 7 min. Amplicons were analyzed via 1.5% agarose gel electrophoresis, purified by magnetic beads technique, and subject to Sanger direct sequencing. The obtained sequences were analyzed using Sequencher® software[4].

RESULTS

Considering the 95% confidence interval, the sample size used in the present study was slightly larger than the minimum required to assess the allele frequency of *SOD1*:c.118G>A variant (122 vs. 113). All animals assessed in this study (122 animals) were clinically normal at the time of sampling and were identified as wild-type for the *SOD1*:c.118G>A variant. Therefore, no alleles of the pathogenic variant (A) were found in the studied population.

DISCUSSION

The present study is the 1st report to evaluate the prevalence of *SOD1*:c.118G>A variant in Brazilian Golden Retriever dogs, in which all samples and animals assessed were classified as wild-type. Although this variant has not been described and detected in the Brazilian Golden Retriever, studies in other countries contributed to defining the importance of this variant in many breeds [3,4,6,9,11] and as a valuable natural model for the study of amyotrophic lateral sclerosis (ALS) in humans [13].

Awano and collaborators were the 1st to describe the *SOD1*:c.118G>A variant in the Boxer, German Shepherd, Chesapeake Bay Retriever, Pembroke Welsh Corgi, and Rhodesian Ridgeback breeds [4]. Later, Zeng and colleagues evaluated the variant in

more than 124 breeds and mixed-breed dogs, suggesting that *SOD1*:c.118G>A is widely spread and fixed in the species before the breeds' origin [18].

Altogether, available data seems to indicate a rare prevalence of *SOD1*:c.118G>A in the Golden Retriever breed. This is supported by the results of this study, where all animals assessed were classified as wild-type for the variant, yielding an allele frequency below 0.01, and the studies performed with Mexican [5], Belgian [6], Dutch [6], German [6], and American [18] Golden Retriever populations further proves it, which found a prevalence ranging from zero [5,6] to 0.03 [18].

There is a possibility that a still undetected genetic variant is a risk factor for CDM in Golden Retrievers, as it was already shown for the Bernese Mountain Dog. This is the only breed where a 2nd risk factor was already detected [18]. To clarify this point, it would be necessary to sequence the entire SOD1 gene, mainly in dogs diagnosed with the disease.

The genetics of the Brazilian Golden Retriever are composed of many blood lineages, mainly from those from the USA [16]. With this in mind, the authors believe that the result observed in this study is because our samples are composed mainly of dogs from the USA blood lineages, where *SOD1*:c.118G>A prevalence found is considered lower than 0.03 [18].

The definitive diagnosis of CDM is performed by post mortem histopathology findings, such as axonal and myelin degeneration and astrogliosis in the spinal cord funiculus [13,18], and genotyping the animals for the variant associated with CDM [4,12,16]. The clinical signs observed in CDM can be found in many other diseases, such as medullary neoplasms, disc protrusion, and hip dysplasia [8] and genetic testing serves a pivotal role in aiding Veterinarians in the differential diagnosis of such disorders sharing similar clinical manifestations [12].

Since CDM has an irrepressible progression and a poor prognosis [3,7,9,13,18], most of the owners opt for early euthanasia of the affected animals [7,18]. The knowledge of the disease by owners and breeders and the genotyping of their animals may prevent the dissemination of the variant among lineages and also help Veterinarians in differential diagnosis and how to conduct cases of CDM.

CONCLUSION

The variant *SOD1*:c.118G>A, responsible for CDM in several dog breeds, was not detected in this Brazilian Golden Retriever population analyzed in this study. The occurrence of the variant seems to be rare in this specific breed, and although it has been reported in Brazil in other breeds, we can not affirm that *SOD1*:c.118G>A does not occur and affects Brazilian Golden Retriever dogs.

MANUFACTURERS

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***Acknowledgments*.** This study was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), grant number 2023/03709-2, and CPNQ (Conselho Nacional de Desenvolvimento Científico e Tecnológico), grant number 140338/2021-7.

***Ethical approval*.** This study was approved on 09 October 2017 by the Institutional Animal Care and Use Committee (205/2017 – CEUA – UNESP), and samples were collected under a strict confidentiality agreement to ensure the anonymity of establishments, owners, and animals.

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

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