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Case Report

Ehlers-Danlos Syndrome in a Mangalarga–Campolina Crossbreed Mare

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ABSTRACT

The Ehlers-Danlos syndrome in horses is a group of genetic connective tissue disorders clinically characterized by skin fragility and hyperextensibility. To date, only two of those conditions (Hereditary Equine Regional Dermal Asthenia and Warmblood Fragile Foal Syndrome [WFFS]) have been characterized based on the causative genetic mutations. This report describes the dermatological and histological findings observed in a 3.5-year-old Mangalarga and Campolina crossbreed mare with recurrent skin wounds. Upon dermatological examination, the mare presented with hyperextensible, fragile, and thin skin areas, and scars distributed mainly along the dorsal regions. Histopathological evaluation of affected skin biopsies revealed collagen fibers abnormalities within the deep dermis. The complete *PPIB* coding region was amplified, but no mutations were observed. Moreover, the *PLOD1* gene mutation responsible for WFFS was not present in this animal. To our knowledge, this is the first report describing a Brazilian non-Quarter horse mare with dermatological and histopathological findings of Ehlers-Danlos syndrome.

1. Introduction

In humans, the Ehlers-Danlos syndrome is a heterogeneous group of heritable connective tissue disorders characterized by skin hyperextensibility, articular hypermobility, easy bruising, and connective tissue fragility [1,2]. These disorders occur due to abnormal collagen biosynthesis as a result of mutations in several genes [3]. In horses, the Ehlers-Danlos syndrome also includes different inherited connective tissue disorders based on the clinical presentation, preferentially affected breed, and causative genetic mutation. Two distinct disorders (i.e., Hereditary Equine Regional Dermal Asthenia [HERDA] and Warmblood Fragile Foal Syndrome [WFFS]) have been characterized based on the causative genetic mutations [4,5].

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Hereditary Equine Regional Dermal Asthenia is an autosomal recessive disease frequently described in the Quarter Horses and related breeds [6–9]. Clinical signs of the disease are usually associated with the beginning of saddle training [8]. Hereditary Equine Regional Dermal Asthenia is caused by a missense mutation (c.115G>A) in the peptidylprolyl isomerase B (*PPIB*) gene [4]. Warmblood Fragile Foal Syndrome is also an autosomal recessive disease, but it affects Warmblood breeds [10–14]. The disease is caused by a missense mutation (c.2032G>A) in the equine procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (*PLOD1*) gene [5]. Although the *PLOD1* mutation has been identified in only one case report [14], there are four other case reports [10–13] published before the identification of the causative mutation where the animals were suspected to be affected by the same condition [14].

In addition, Ehlers-Danlos syndromes in horses were described in a few previous reports [15–17]. In those reports, a 2-year-old Arabian cross filly [15], a 6-year-old Thoroughbred gelding [16], and a 7-year-old Quarter Horse gelding [17] developed open wounds and atrophic scars as a result of skin fragility. Furthermore, those horses presented hyperextensible skin, which was most evident over the shoulders and dorsum. Although the clinical signs of the disease reported in those case reports resembled both HERDA and WFFS, the disorder's cause (genetic or otherwise) was not determined. Besides HERDA [9], other Ehlers-Danlos syndromes affecting horses have not been previously identified in Brazil. This report describes the dermatological and histological findings and the molecular characterization of the complete *PPIB* coding region in a Mangalarga and Campolina crossbreed mare affected with Ehlers-Danlos syndrome.

2. Case Presentation

A 3.5-year-old Mangalarga and Campolina crossbreed mare was referred for evaluation of recurrent skin wounds over the back that had been present for over 1 year. The owner also reported fragile and elastic skin along the body. The owner was unaware of any traumatic incident that could have caused the wounds. The mare had been purchased as a foal at an auction with no apparent skin abnormalities.

At presentation, the mare was in good body condition. Physical examination revealed bilateral asymmetric skin lesions distributed mainly on the neck and along the dorsal regions. The abnormalities consisted of fragile and thin skin, scarring, and tumor-like masses. The abnormal skin was also hyperextensible when pinched up (stretching about 10 cm above the skin surface), slowly returning to its normal position upon release. Marked leukotrichia was observed over the right shoulder on an area that had been previously wounded (Fig. 1). Besides the dermatological findings, no evident ocular and musculoskeletal abnormalities were observed.

Incisional biopsies were performed on the affected areas of the neck and dorsal loin region after local

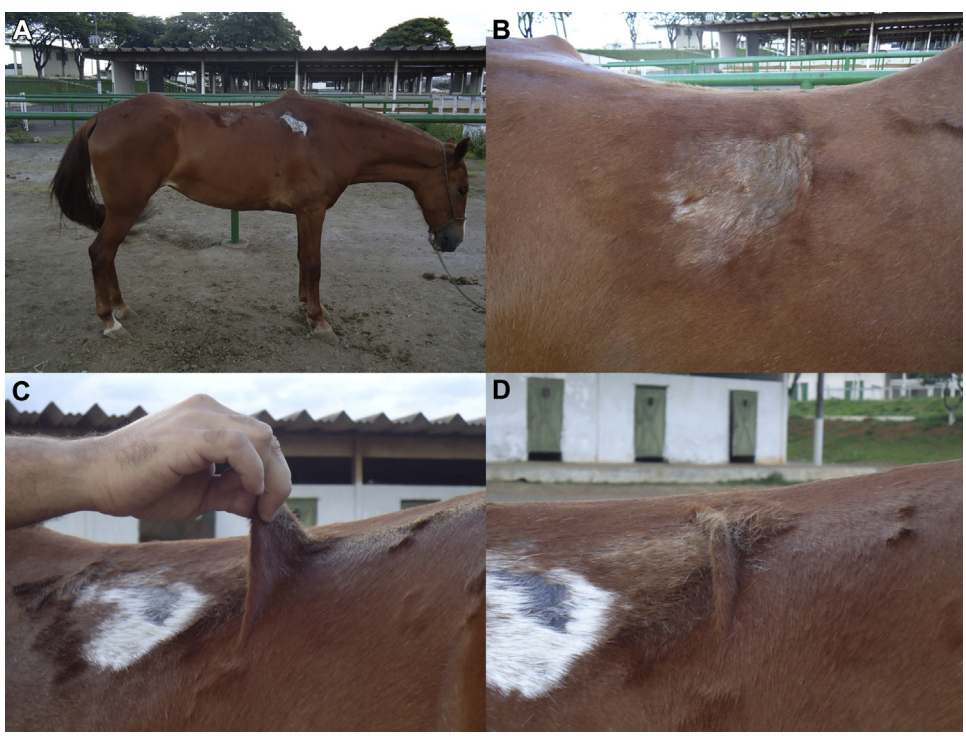

Fig. 1. (A) Mangalarga and Campolina crossbreed mare with Ehlers-Danlos syndrome. (A) Right side view of the whole body. (B) Healed lesion at the right side presenting leukotrichia. (C) Marked cutaneous hyperextensible (stretched 10 cm above the skin surface) on the left dorsum area. (D) Hyperextensible skin slowly returned to its original position.

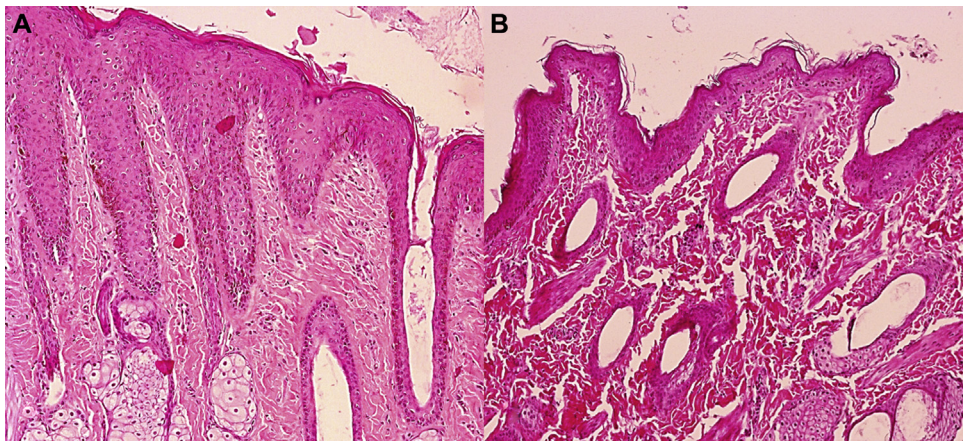

Fig. 2. Histological appearance (H&E) of the skin in mare affected with Ehlers-Danlos syndrome. (A) Control Quarter Horse. (B) Affected mare. Note the loosely arranged thin, small, and fragmented collagen bundles within the superficial and perifollicular dermis in the affected skin.

anesthesia with lidocaine 2% (Xylestesin, Cristália, Itapira, São Paulo, Brazil). The skin samples were routinely processed. Histopathological evaluation of the skin specimens revealed thin, small, and fragmented collagen fibrils that created a mild loose arrangement of the collagen fibers within the superficial and perifollicular dermis (Fig. 2). Mild perivascular mononuclear infiltrate was found in the superficial dermis. The epidermis and epidermal adnexa were unaffected. The presumptive diagnosis of Ehlers-Danlos syndrome was made based on the history, dermatological signs, and histopathological examination.

DNA was purified from whole blood using the Ilustra blood genomic Prep Mini Spin Kit (GE Healthcare Life Science, Little Chalfont, Buckinghamshire, UK). The mare was tested for both HERDA and WFFS causative mutations. The missense mutation in the *PPIB* gene was evaluated as previously described [18]. *PLOD1* gene mutation was evaluated at Cornell University [5]. The sequences were analyzed, and the mutations were not confirmed. Then, we performed the characterization of the complete *PPIB* coding region in order to observe any mutation that could be related to the disease. Primer sets were designed with the Primer Express software (Applied Biosystems, Grand Island, NY) (Table 1). The provisional *PPIB* gene sequence (Gene ID: 100066834) of the equine reference genome assembly (EquCab2.0) available in the NCBI GenBank was used to design the primer sets. The exon 1 forward primer and the exon 5 reverse primers were anchored in the 5′ and 3′ untranslated regions of the *PPIB* gene, respectively.

The 50 μL polymerase chain reaction contained 25 μL of GoTaq Green PCR Master Mix (Promega, Madison, WI), 0.3 μM of each forward and reverse primer, 5 μL of template DNA, and nuclease-free water to complete the final volume. The amplification conditions were as follows: initial denaturation at 95°C for 2 minutes; followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes. Amplicons were analyzed by 1.5% agarose gel electrophoresis, purified, and submitted to direct sequencing. The obtained sequences and electropherograms were analyzed using Sequencing Analysis 5.3.1 software (Applied Biosystems, Grand Island, NY) and aligned against the reference genome sequence. Homology differences were not observed between the mare and wild-type *PPIB* coding region sequences.

Table 1
Primer sets used for the peptidylprolyl isomerase B coding region characterization.

Primer Set	Sequence (5′—3′)	Product (bp)	Fragment Amplified
P1	Forward—CCCGCGGGGGACCTACTATT Reverse—GGCTGAGGTGTCCGGGTCAT	521	UTR5 and exon 1
P2	Forward—TTCTCCCGGTGGATGCTGCGTTT Reverse—TTTGCAACAGACAGCACTGAGGC	420	Exon 1
P3	Forward—CGTAGTCTGAAGGAGAGTGGCAAA Reverse—AACAGTTAGCTGGTACCCAGTGAG	465	Exon 2
P4	Forward—ATAATTCTGGCCAGCAGCAGTGTC Reverse—TACAAAGAAGGGTGCAGCTGGTCT	372	Exon 3
P5	Forward—ACACTTGGTCAGCATGGTTTGGCT Reverse—GAACAATGGTCTGAATCTGTGGGT	498	Exon 4
P6	Forward—CCTCAAGGCTTGTATGCTGCTTGT Reverse—AAACTGGGCCCTGTGGAATGTGA	410	Exon 5
P7	Forward—CACTGGGGCCTGCCTCTCTT Reverse—TGGGGAATGGGTTCAGGCCA	535	Exon 5 and UTR3

3. Discussion

The clinical history, dermatological signs, histopathological examination, and the absence of the HERDA and WFFS causative mutations are consistent with a diagnosis of Ehlers-Danlos syndrome. To date, *PPIB* and *PLOD1* causative mutations allow equine practitioners to differentiate between HERDA [4] and WFFS [5]. Cases that are not diagnosed as one of those diseases are commonly referred as Ehlers-Danlos syndrome or simply a disorder of collagen [15–17]. The clinical and histopathological findings of these disorders are quite similar [8,9,13,14,19], making the differential diagnosis difficult with no genetic information. However, clinical presentation, age of onset of dermatological findings, and affected breed usually help to differentiate between HERDA and WFFS.

Hereditary Equine Regional Dermal Asthenia–affected horses exhibit dermatological lesions with 1.3 years of age after the beginning of saddle training, and the lesions are mainly distributed along their dorsum [8], which are the area of the body most exposed to sunlight [20]. Similarly, lesions in horses affected with Ehlers-Danlos syndrome occur on the shoulders and dorsal areas, but the age of onset of dermatological signs varies between 2 and 7 years [15–17]. Typically, WFFS affects neonates and juvenile horses up to 6 weeks of age usually presenting hematomas, multiple lacerations, and skin fragility in the flanks, limbs, perineum, and abdomen [10–14]. However, a previous report described a 1.5-year-old Swiss Warmblood filly with lesions distributed in the flank and along the back, which was tested negative for the *PPIB* mutation causative of HERDA [12] and the *PLOD1* mutation causative of WFFS [5]. The clinical presentation and age of onset of dermatological signs in the present case report were very similar to those observed in HERDA [8], the case of the Swiss Warmblood filly [12], and previous cases of Ehlers-Danlos syndrome [15–17].

Horses affected with HERDA exhibit increased incidence of corneal ulcers, mild corneal opacity, decreased corneal thickness, increased curvature diameter of the cornea, and multifocal corneal collagen fibers disorganization [21,22]. Moreover, HERDA-affected horses show profoundly articular hypermobility and weaker tendinoligamentous tissues [23,24]. The mare reported here did not exhibit articular hypermobility or ocular abnormalities such as opacity, ulcer, and evident scar of the cornea. This animal was a single case diagnosed in a rural area with no interest of a more detailed examination by the owner. Hence, we did not measure the thickness, curvature, or diameter of the cornea, did not evaluate ocular tissue histological sections, and did not perform ultrastructural and biomechanical evaluations of the tendons or ligaments. Therefore, we cannot rule out the possibility of clinically nonevident ocular findings or abnormal physical weakness in collagen-rich tissues.

Unlike HERDA and WFFS, which are described in the Quarter Horse and Warmblood breeds [6,8,13,14], the mare described here was a crossbreed of the Mangalarga and Campolina breeds. In the 18th century, those breeds were originated in Brazil from breeding Portuguese Alter real and the Spanish Andalusian horses with native horses [25]. The Mangalarga and Campolina breeds have no known Quarter Horse or Warmblood ancestors [25], which could segregate the causative allele mutations. Therefore, it is not surprising that *PPIB* and *PLOD1* mutations tested negative in the mare reported here. In addition, the probable inheritance pattern of the condition could not be performed in this case report because the owner did not have any information besides the breeds of origin.

In humans, distinct mutations in the same gene may cause similar clinical phenotypes of Ehlers-Danlos syndrome [2]. Despite the negative results for *PPIB* and *PLOD1* causative mutations, the case described in the present report resembled HERDA regarding the age of onset, dermatological signs, and localization of the lesions. Therefore, we decided to perform the entire characterization of the equine *PPIB* coding region. Although no mutation was observed in the characterized *PPIB* coding region, other candidate genes were not investigated because the case reported was an isolated diagnosis. It is possible that, similar to the disease in humans, the Ehlers-Danlos syndrome in horses is a group of several diseases caused by mutations in several genes and/or distinct mutations in the same gene [17]. Therefore, despite being clinically distinct from WFFS, we cannot rule out another mutation in *PLOD1* as the causative of the phenotype described. It is also possible that the present case might be the result of novel and spontaneous mutation as previously suggested [17].

In summary, this is the first report in Brazil describing a horse affected with Ehlers-Danlos syndrome which clinically resembled HERDA, but without a *PPIB* mutation. The disease might be associated with novel mutations in the *PLOD1* coding region or in another gene involved in the collagen biosynthesis rather than *PPIB*.

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