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Ehlers-Danlos syndrome in a crossbreed cat

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ABSTRACT: *The Ehlers-Danlos syndrome (EDS) consists of a group of diseases characterized by defective collagen production or failure in its organization, resulting in changes in the strength and extensibility of connective tissue. This report describes the dermatological and histological findings observed in a 3-month-old crossbreed cat with rupture and detachment of skin in the thoracic limb and rupture of the skin in the cervical region. Upon dermatological examination, the cat presented fragile and hyperextensible skin in the cervical region and a skin extensibility index of 21%. Histopathological evaluation of the skin specimens revealed evident disorganization of collagen bundles in dermis and in the Masson's trichrome staining, follicular dysplasia was found. The presumptive diagnosis of EDS was made based on the clinical and histopathological findings. Sanger sequencing did not detect any mutated alleles for the c.3420delG mutation in COL5A1 gene, which was an autosomal dominant mutation previously been associated with Ehlers-Danlos syndrome in cats. The absence of this mutation in the reported cat suggests that other mutation may also be responsible for the development of cutaneous asthenia in this or maybe other genes related to collagen metabolism.*
Key words: *collagen, dermatology, laceration, mutation, sequencing.*

Síndrome de Ehlers-Danlos em um gato mestiço

RESUMO: *A síndrome de Ehlers-Danlos (EDS) consiste em um conjunto de doenças caracterizadas pela produção deficiente de colágeno ou falha em sua organização, resultando em alterações na resistência e extensibilidade do tecido conjuntivo. Este relato descreve os achados dermatológicos e histológicos observados em um gato mestiço de três meses de idade com ruptura e descolamento de pele do membro torácico e ruptura da pele na região cervical. Ao exame dermatológico, o gato apresentava pele hiper-extensível, fragilizada na região cervical e índice de extensibilidade cutânea de 21%. A avaliação histopatológica das amostras de pele revelou desorganização evidente dos feixes de colágeno na derme e pela coloração com tricrômico de Masson foi encontrada displasia folicular. O diagnóstico presuntivo de EDS foi realizado com base nos achados clínicos e histopatológicos. O sequenciamento de Sanger não detectou nenhum alelo mutado para a mutação c.3420delG no gene COL5A1, que é uma mutação autossômica dominante previamente associada à síndrome de Ehlers-Danlos em gatos. A ausência dessa mutação no gato relatado sugere que outra mutação também pode ser responsável pelo desenvolvimento de astenia cutânea neste gene ou em outro associado ao metabolismo de colágeno.*
Palavras-chave: *colágeno, dermatologia, laceração, mutação, sequenciamento.*

Cutaneous asthenia, referred to as Ehlers-Danlos Syndrome (EDS) in human medicine, consists mof a set of diseases characterized by defective collagen production or failure in its organization, resulting in changes in the strength and extensibility of connective tissue (HANSEN et al., 2015). Considered rare in cats (SEQUEIRA et al., 1999; BENITAH et al., 2004; SZCZEPANIK et al., 2006; DOKUZEYLUL et al., 2013; HANSEN et al., 2015; SEO et al., 2016; SPYCHER et al., 2018), the clinical signs are diverse and related to hyperextensibility and joint laxity, in addition to fragility cutaneous, with less resistance to traction and tendency to sag (DOKUZEYLUL et al., 2013; HANSEN et al., 2015).

Of genetic origin, this syndrome is related to mutations in the genes that encode the collagen or enzymes responsible for its organization. In humans, several forms of this syndrome have already been

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described, based on the clinical presentation, affected tissue and molecular characterization (HANSEN et al., 2015). In cats, however, only two forms have been described, being one characterized by deficiency in the enzyme procolagene-N-peptidase type 1 (COUNTS et al., 1980) and another related with autosomal dominant origin, similar to types 1 and 2 of humans (PATTERSON & MINOR, 1977).

In cats, it is known that the deletion of a single base pair in exon 43 in the *COL5A1* gene (c.3420delG) is related to the development of EDS (SPYCHER et al., 2018). Therefore, the present study described the dermatological and histological findings observed in a 3-month-old crossbreed cat with clinical and histological findings compatible with EDS.

A male cat, crossbred, with an estimated age of 3 months, was abandoned in a veterinary clinic. The animal was in good general condition and wascastrated without any complications, however, one of the kittens started to present recurrent skin lesions in the cervical region, in the posterior part of the auricles, in the back and in the limbs, after licking or scratching the body. The lesions were characterized by skin laceration, with apparent subcutaneous tissue and little bleeding (Figure 1A, B, C). In addition, flaccidity and hyperextensibility of the animal's skin was observed. The fragility of the skin became more evident during physical restraint to collect blood by puncture the jugular vein. There was tearing and detachment of the skin on the thoracic limb and rupture of the skin on the cervical region, at the puncture and pressure site at the withers.

To exclude parasitological and fungal causes, a parasitological examination was performed by skin shaving and fungal hair culture collected around the lesions, and both had negative results. The most extensive lesions were sutured, but there was worsening with the formation of granulomas and healing occurred by second intention, in a longer time than expected (approximately 50 days). The scars formed were atrophic, forming a depression in the skin, which had a thin, smooth, whitish, and shiny appearance.

Due to the suspicion of cutaneous asthenia, the skin extensibility index (SEI - Skin Extensibility Index) was calculated (SEI = height of the skin over the lumbar region in cm ÷ distance from the occiput to the base of the tail in cm x 100), obtaining if result of 21% (reference: ≤19%) (Szczepanik *et al*., 2006).

Histopathological examination was performed (Figure 1 D, E), which revealed a preserved epidermis, with a thin and delicate aspect. At the interface, intense edema of collagen fibers was observed, associated with the presence of discrete individualized mast cells in the superficial dermis to the deep dermis. Collagen fiber dysplasia was noted, showing disorganization of the collagen bundles. The attachments were intact and preserved. After Masson's trichrome staining, irregularity of collagen fibers was observed, with loss of cohesion and alteration of collagenous spacing, showing the condition of follicular dysplasia.

To search for the c.3420delG mutation in COL5A1 gene, Sanger sequencing was performed using blood DNA sample and COL5A1_F1 (AAGCTGGCTGAAACCCATC) and COL5A1_R1 (CGAGCACTCCAGAGATGTCA) primers previously described (SPYCHER et al., 2018). However, the presence of the mutation was not detected in the analyzed fragment (Figure 1F).

Management measures were established to avoid trauma and the appearance of new injuries, such as: indoor lifestyle, use of clothes and Elizabethan collar, maintenance of trimmed nails and periodic control of ectoparasites. Currently, the cat is 6 years and 4 months old and, according to the tutor, has a good quality of life following the recommendations mentioned.

Ehlers-Danlos syndrome, or cutaneous asthenia, is a rare inherited disease of connective tissue, characterized by hyperextensibility and laxity of the skin (DOKUZEYLUL et al., 2013). There are reports of different cat breeds affected by this syndrome, such as the Himalayas (COUNTS et al., 1980; HOLBROOK et al., 1980), Birman (SZCZEPANIK et al., 2006) and Burmese (HANSEN et al., 2015). However, as in the present report, most animals are crossbreed (BENITAH et al., 2004; SZCZEPANIK et al., 2006; DOKUZEYLUL et al., 2013; SEO et al., 2016).

The cat in the present report was four months old when the first clinical signs were observed, similar with previously report (HANSEN et al., 2015), in which in this species, the onset of clinical signs varies from a few months to 2 years. The characteristics of the observed lesions have also been described (HANSEN et al., 2015) and included skin lacerations in the cervical region, with the formation of atrophic and thin scars. In most cases, the integumentary system is the only one affected, where the skin is thin, delicate and hyperextensible (SZCZEPANIK et al., 2006). However, there is a case report of a cat with systemic impairment (BENITAH et al., 2004).

The diagnosis is made based on clinical signs, associated with the skin extensibility index (HANSEN et al., 2015) and histopathological findings (SEO et al., 2016). In cats, the extensibility index above 19% is considered abnormal and,

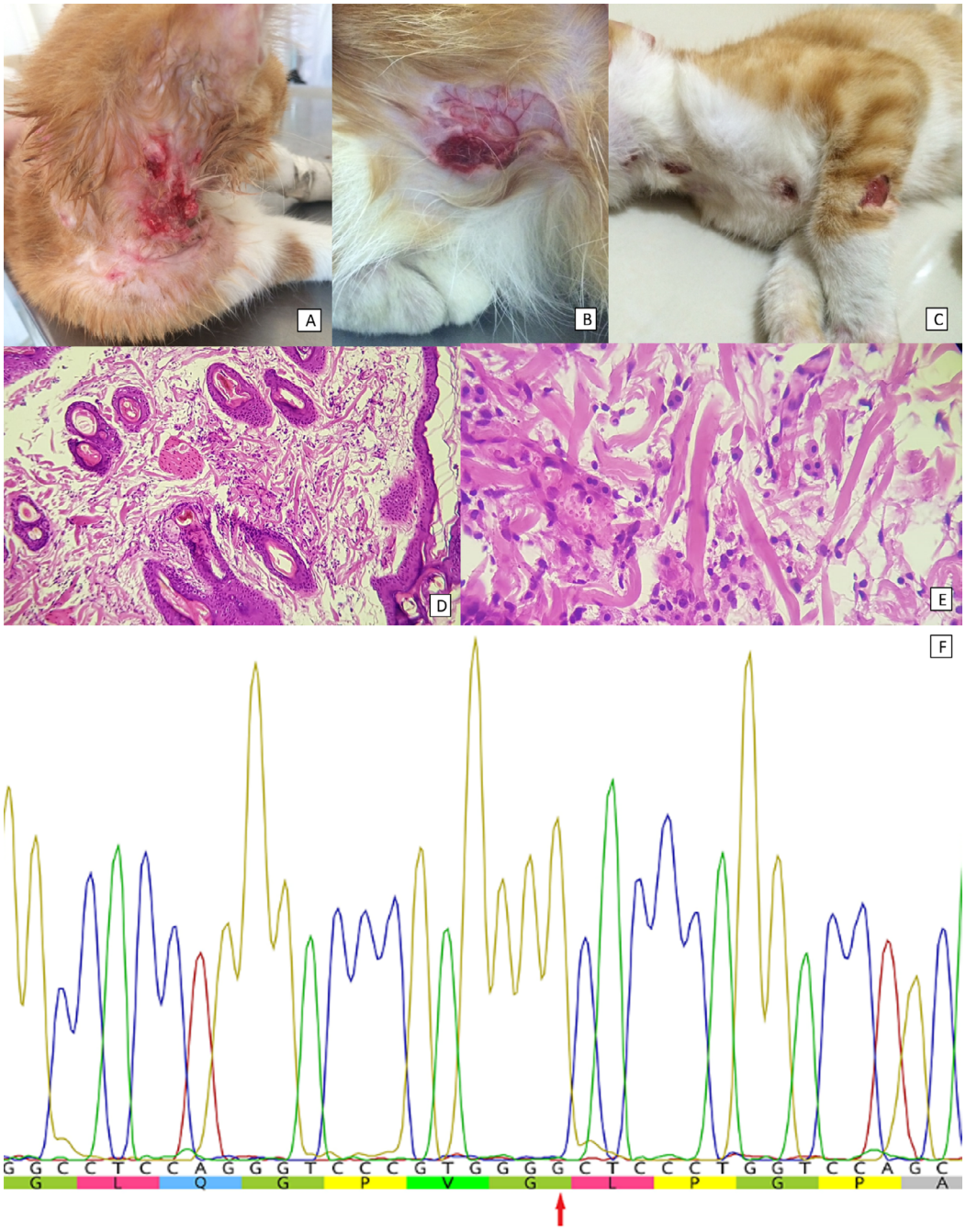

Figure 1 - (A) Laceration in the cervical region due to physical restraint. (B) and (C) Injuries resulting from restraint and jugular puncture for blood collection. (D) Histopathological analysis of a skin fragment with hematoxylin-eosin stain showing preserved epidermis, with fine and delicate appearance. At the interface, intense edema is observed associated. The skin appendages are intact and preserved (40x). (E) Edema and dysplasia of collagen fibers is observed showing disorganization of the collagen bundles (200x). (F) Partial chromatogram showing capillary sequencing results for homozygous wildtype of the c.3420delG mutation in COL5A1 gene in crossbred cat with Ehlers-Danlos syndrome. Note the mutation site (red arrow), where a deletion of a single guanine leading to a frameshift previously associated with Ehlers-Danlos syndrome in cats was not observed. Image obtained using Geneious® 10.0 software (Biomatters Ltd., Aucklans, New Zealand).

therefore, hyperextension (SZCZEPANIK et al., 2006; GONDIN & ARAUJO, 2020). In the animal described, the index obtained was 21%.

In the histopathological analysis of a skin fragment with hematoxylin-eosin, a reduction in the number of collagen fibers is observed, in an irregular pattern, in addition to thinning and folding of the epidermis (SZCZEPANIK et al., 2006), as observed in the present report. When Van Gieson's stain is used, irregular patterned collagen fibers are observed, fragmented, and separated by irregular spaces (SZCZEPANIK et al., 2006). Masson's Trichrome staining helps the diagnosis, where, in animals with cutaneous asthenia, abnormalities in the staining of collagen fibers are evident. In this report, the use of this technique showed irregularity of collagen fibers, with loss of cohesion and alteration of collagenous spacing. FERNANDEZ et al. (1998), using this stain, demonstrated that all 8 animals with cutaneous asthenia had abnormalities in the collagen fibers, but the cause of the staining abnormality in this condition is still unclear.

Already reported in dogs (BAUER et al., 2019a), the only described mutation related to this disease in cats was in the COL5A1 gene (SPYCHER et al., 2018). This gene provides instructions for the synthesis of the pro-alpha 1 chain, which makes up type V collagen (NICHOLLS et al., 1996). However, due to its dominant inheritance, the c.3420delG mutation may not be reported in all cases (SPYCHER et al., 2018). The absence of this mutation in the cat in this report showed that more than one genetic mutation may be associated with the development of the syndrome in this species.

Other mutations related to this syndrome in animals have already been identified in ADAMTS2, TNXB and EPYC genes. The ADAMTS2 gene, responsible for the N-proteinase enzyme synthesis instruction, and it is related to the organization of the collagen chains in functional fibrils (BEKHOUCHE & COLIGE, 2015). In the mutated gene, a nucleotide codon is replaced by a premature termination codon, which results in abnormal collagen fibers, having already been described in dogs, sheep, and cattle (COLIGE et al., 1999; ZHOU et al., 2012; JAFFEY et al., 2019).

In dogs, the involvement of the TNXB gene in Ehlers-Danlos syndrome has also been described (BAUER et al., 2019b). This gene is associated with the production of the Tenascin-X glycoprotein, responsible for organizing and maintaining the body's supporting tissue structures, such as muscles, joints and skin (BAUER et al., 2019b). Despite the hypermobility and extensibility of the skin also present, the main feature is the non-development of atrophic scars, which are common in the classic form of the syndrome (BAUER et al., 2019b).

On the EPYC gene mutation described in cattle, the replacement of a guanine with an adenine occurs in the nucleotide position 254, leading to the absence of dermatan sulfate proteoglycans in the connective tissues (TAJIMA et al., 1999) Thus, a clinical picture similar to Ehlers Danlos syndrome is presented by the animal, with delayed wound healing, soft, fragile and hyper-extensible skin (TAJIMA et al., 1999).

The most reported genetic inheritance pattern in Ehlers-Danlos syndrome in cats is the dominant type; however, there are reports of recessive inheritance (HOLBROOK et al., 1980; HANSEN et al., 2015). In our report, there was only one animal in the litter affected by the condition, in addition to the mother also not having hyperextensibility and skin fragility, which reinforces the suspicion in the mentioned pattern of inheritance.

Although, the prognosis is considered poor, since there is no specific treatment protocol for this condition (SZCZEPANIK et al., 2006; DOKUZEYLUL et al., 2013), the animal in the present report has a quality survival of more than 6 years of life, maintaining management care to avoid skin lesions, in addition to restricted access to the street.

Cutaneous asthenia should be a differential diagnosis in cats with skin lacerations. The molecular examination alone may not be sufficient as a diagnostic method in all cases, since other mutations, in addition to the c.3420delG described in the COL5A1 gene, may be responsible for the development of cutaneous asthenia in cats. Management measures should be instituted to avoid traumatic injuries, such as indoor lifestyle, use of clothes and Elizabethan collar, maintenance of trimmed nails and control of ectoparasites.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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