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Neoplastic disease

# Feline osteochondromatosis in a 12-year-old feline leukaemia virus-negative cat

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ABSTRACT

Feline osteochondromatosis is a spontaneous osteocartilaginous exostosis associated with feline leukaemia virus (FeLV) infection or due to a frameshift variant in the exostosin glycosyltransferase 1 (*EXT1*) gene. Osteochondromatosis was diagnosed in an indoor-only, 12-year-old, neutered female, Russian Blue cat. Radiographs revealed bilateral calcified proliferations in the elbow, costochondral and sternochondral joints, which distorted the normal skeletal structure. Grossly, the proliferated joints presented with consistent, rounded masses, causing complete ankylosis. The main histopathological finding was an osteocartilaginous proliferation composed of multiple irregular islands of well-differentiated hyaline cartilage surrounded and delimited by osteoid tissue. Immunohistochemistry of the osteochondromas, bone marrow and mediastinal lymph nodes, using a primary anti-FeLV gp70 antibody, and FeLV proviral DNA real-time polymerase chain reaction on bone marrow were negative. Sequencing of exon 6 of the *EXT1* gene was performed and nucleotide BLAST analysis demonstrated the absence of a frameshift variant. This study reports the only case of spontaneous feline osteochondromatosis in an animal more than 10 years old.


The term osteochondroma defines bony outgrowths covered by cartilage that form in the metaphysis of bones with endochondral ossification. These outgrowths present as either solitary osteochondromas or osteochondromatosis (also called multiple cartilaginous exostosis, polyostotic osteochondroma or hereditary multiple exostosis).

Osteochondromatosis has been reported in humans [1], horses [2], dogs [3], mice [4] and cats [5] and has been linked to exostosin glycosyltransferase 1 and 2 (*EXT1* and *EXT2*) gene mutations as a result of an autosomal dominant disorder. Generally, osteochondromatosis only appears up to epiphyseal plate closure, but in cats osteocartilaginous growth can be observed even after bone maturity, indicating aetiologies other than autosomal genetic disorders. Feline osteochondromatosis usually occurs in adult cats with skeletal maturity [6]. There is no breed or sex predisposition and incidence is uncertain; one study reported a 20% prevalence in a population of 15 feline primary bone tumours [7]. Feline osteochondromatosis can be spontaneous [8,9], due to a frameshift variant in exon 6 of the *EXT1* gene [5], or be associated with feline leukaemia virus (FeLV) [10,11]. The complex diagnosis of various types of FeLV infection (progressive, regressive, abortive and focal) hinders the association with feline osteochondromatosis.

We now describe the radiographic and pathological findings in a case of feline osteochondromatosis. To evaluate the aetiology, FeLV immunohistochemistry (IHC), real-time polymerase chain reaction (RT-PCR) and sequencing of exon 6 of the *EXT1* gene were performed.

An indoor-only, 12-year-old, neutered female, Russian Blue cat presented at 10 years of age with a forelimb lameness that worsened over the following years. The most severely affected joints were the elbows, which were increased in size, leading to complete ankylosis. The cat's condition progressively deteriorated and it died spontaneously.

Prior to necropsy, radiographic examination revealed bilateral calcified proliferations at the elbow joints, vertebral bodies T7–T10 and L2–L3 and the costochondral and sternochondral joints of the 9th and 10th left ribs (Fig. 1). These proliferations deformed the

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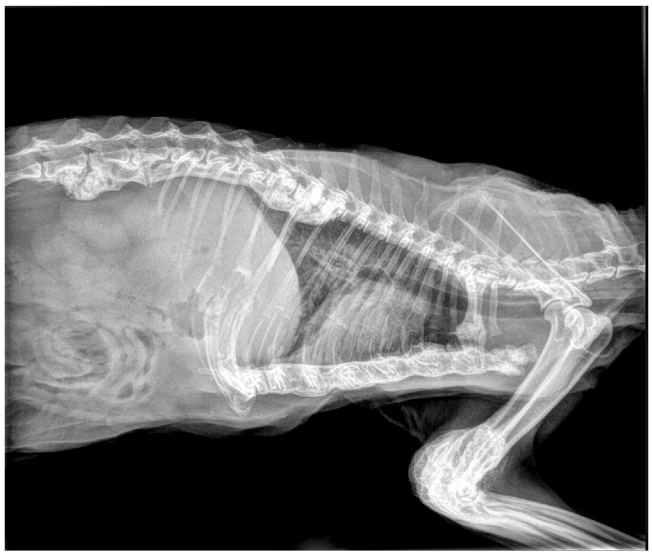

**Fig. 1.** Feline osteochondromatosis, laterolateral radiograph, cat. Calcified proliferations compatible with osseous neoplasms in elbows, vertebral bodies and costochondral and sternochondral joints deform normal skeletal structure.

normal skeletal structure and were compatible with osseous neoplasms. A moth-eaten radiographic pattern was observed, in which radiodense grooves were compatible with cancellous bone and radiolucent areas corresponded to non-ossified cartilage.

Grossly, each elbow joint presented with a consistent rounded mass (6 × 5 cm diameter) that caused complete ankylosis (Fig. 2). On section, an irregular, whitish osteoid proliferation replacing normal tissue was observed. Lesions in the vertebral bodies and the costochondral and sternochondral joints had similar characteristics. Samples of the damaged joints were fixed in 10% buffered formalin, decalcified with a 35–40% dilution of hydrochloric acid (Rapid Decalcifier; ThermoFisher Scientific, www.thermofisher.com) for 24 h, embedded in paraffin wax, cut at 4 μm and stained with haematoxylin and eosin (HE). Microscopically, expanding and disrupting the bone metaphysis, there was an osteocartilaginous proliferation composed of multiple irregular islands of well-differentiated hyaline cartilage surrounded and delimited by cancellous bone (Fig. 3). Multifocally, proliferated chondrocytes also formed from the overlying periosteum (Fig. 3). Bone marrow was hypocellular and fibrotic (Fig. 3).

Additional sections of the osteochondroma masses, bone marrow and mediastinal lymph nodes were subjected to IHC using an anti-FeLV gp70 primary antibody (AbD Serotec, www.bio-rad-antibodies.com). Endogenous peroxidase was blocked with 0.03% hydrogen peroxide for 5 min. Subsequently, the sections were subjected to antigen retrieval by incubation in a pressure cooker for 3 min in citrate buffer pH 6.0. Anti-FeLV gp70 antibody was diluted 1:640 in phosphate buffered saline (PBS) and the tissue sections were incubated for 30 min at room temperature. Labelling of FeLV was visualized with EnVision + System-HRP Labelled Polymer Anti-mouse (Dako, www.agilent.com); sections were counterstained with haematoxylin. Positive controls included tissue sections from a FeLV-infected cat. Blocks of paraffin-embedded bone marrow were deparaffinized, total nucleic acid was extracted and RT-PCR was performed using primers and probes specific for exogenous FeLV proviral DNA (FeLV RealPCR; IDEXX, www.idexx.es). The immunohistochemical and RT-PCR studies were consistently negative.

For genetic analysis of exon 6 of the *EXT1* gene, DNA was extracted from a paraffin-embedded tissue sample. After dewaxing, a microwave treatment method was applied [12]. The sample was then incubated with proteinase K for 48 h at 56°C, followed by organic purification (phenol:chloroform:isoamyl alcohol, 25:24:1) and a clean-up system procedure (Wizard SV Gel & PCR Clean-Up System; Promega, www.promega.com). To amplify exon 6, the primer pair fEXT1_6 F and fEXT1_6 R (forward primer: 5′-TGCTAGGAGGGAACATTAGCTTAGG-3’; reverse primer: 5′-GCATGACGTTAGACGAGTCCCA-3′) were used as described [5]. PCR was conducted with an annealing temperature of 62.6°C and a final $Mg^{++}$ concentration of 1.5 mM. The resulting PCR product, spanning 343 base pairs (bp), was sequenced. To compare the obtained sequences with GenBank, a nucleotide BLAST (blastn) analysis was performed with a 310 bp good quality sequence. Blastn alignment demonstrated the absence of the single nucleotide duplication (Supplementary material) linked to feline osteochondromatosis [5].

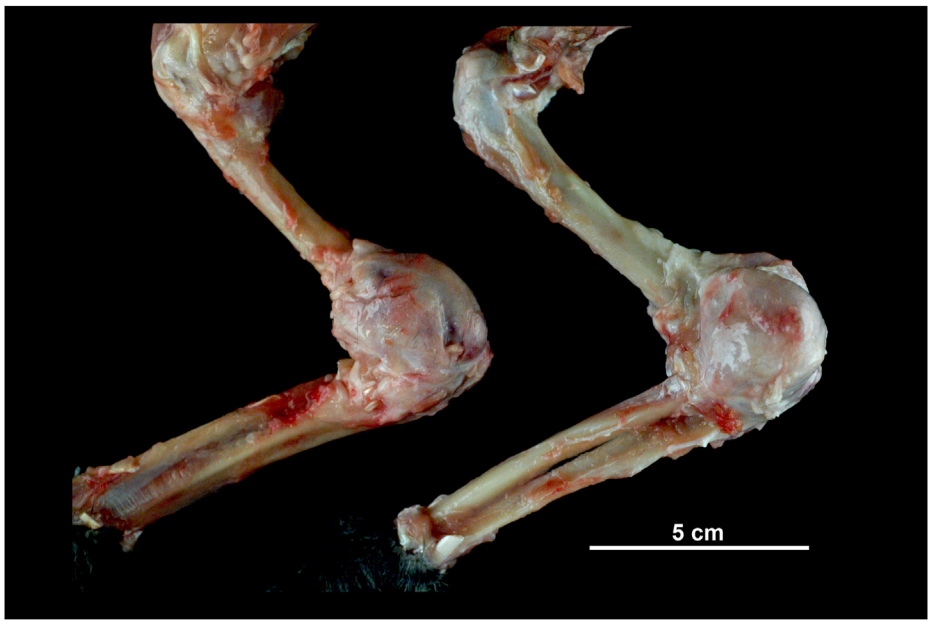


**Fig. 2.** Feline osteochondromatosis, elbow joints, cat. Elbow joints have hard, rounded masses (5 × 6 cm in diameter) causing complete ankylosis.

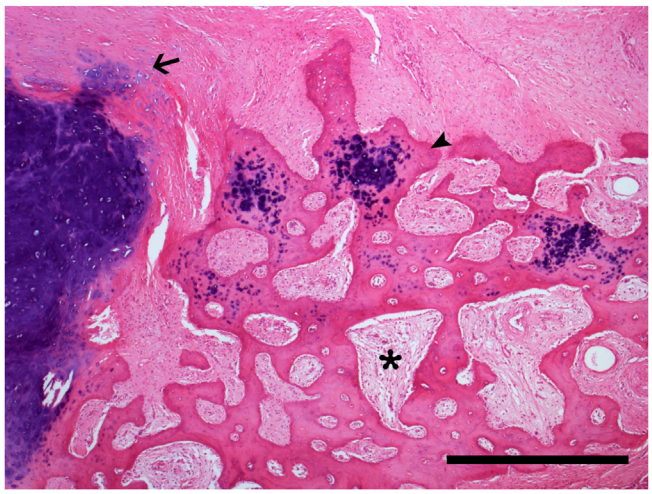

**Fig. 3.** Feline osteochondromatosis, elbow joint, cat. Multiple irregular islands of well-differentiated hyaline cartilage surrounded and delimited by osteoid tissue (arrow). Osteochondromatous areas formed from overlying periosteum (arrowhead). Medullary fibrosis (*). HE. Bar, 1,000 μm.

This study represents the only published case of osteochondromatosis in a cat over 10 years old. This condition of multiple cartilaginous exostoses is rare in cats and is related to a frameshift variant in exon 6 of the *EXT1* gene [5] or to FeLV infection. Sequencing of this exon demonstrated the absence of genetic anomalies [5]. Additionally, FeLV IHC and RT-PCR in this animal were negative. These results could indicate either that the cat had never been infected by FeLV or had an abortive FeLV infection (ie, had cleared the infection). To differentiate between these two possibilities, analysis of anti-FeLV antibodies would have been necessary [13].

Retrovirus-like particles and FeLV genetic material have been found in feline osteochondromas [10,11] but there are three reported cases of feline osteochondromatosis with a negative FeLV status. In one case it was postulated that an *EXT1* variant may have contributed to the osteochondromatosis through loss of function of the EXT1 protein [5]. In the other two cases, the only diagnostic technique used was a serum ELISA against p27 FeLV antigen [8,9]. However, it has been shown that regressively infected cats are negative in routinely used FeLV antigen tests [14]. Interestingly, FeLV causes neoplasia, even in a regressive infection, due to insertional mutagenesis: the virus can activate proto-oncogenes (such as *c-myc* or *flit-1*) or disrupt tumour suppressor genes [14,15], inducing the development of neoplasms without the presence of viral particles or FeLV genetic material intratumourally. To exclude FeLV infection as a potential cause of feline osteochondromatosis, RT-PCR for exogenous FeLV proviral DNA needs to be performed on bone marrow.

In conclusion, we report a case of feline osteochondromatosis in a cat without genetic alterations in exon 6 of the *EXT1* gene or progressive or regressive FeLV infection. This finding reinforces the hypothesis that feline osteochondromatosis can occur spontaneously in aged individuals. Comprehensive diagnosis in cases of feline osteochondromatosis needs to include a variety of tests such as bone marrow and plasma FeLV proviral DNA RT-PCR, together with ELISA for p27 FeLV antigen and anti-FeLV antibodies to confirm or exclude FeLV infection. In animals with a negative FeLV status, genetic analysis should be performed to confirm *EXT1* gene mutation.

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## Statement of author contributions

**Á. Gómez:** Conceptualization, Methodology, Investigation, Resources, Visualization, Writing – original draft, review & editing. **A. Rodríguez-Largo:** Investigation, Resources. **E. Pérez:** Investigation, Resources. **N. Calvo-Sánchez:** Investigation, Resources. **S. Loomans:** Methodology. **K. Chiers:** Methodology. **L. Monteagudo:** Investigation, Resources, Methodology, Writing. **L. Luján:** Investigation, Resources, Methodology, Writing – review & editing. **M. Pérez:** Investigation, Resources, Writing – review & editing, Supervision.

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## Declaration of Competing Interests

The authors declared no conflicts of interest in relation to the research, authorship or publication of this article.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2023.07.003.

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