Feline Leukemia Virus (FeLV) outbreak in Iberian lynxes: proviral *env* sequence analysis and endogenous FeLV quantification

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BACKGROUND:

The Iberian lynx (*lynx pardinus*) is considered the most endangered felid species in the world. Thirteen of 77 free-ranging lynxes tested during the last four years were positive for Feline Leukemia Virus (FeLV) provirus, 11 of them for FeLV p27 antigen as well. All 13 animals tested negative for other viral infections including feline immunodeficiency virus and canine distemper virus. One of the antigenemic lynxes was road-killed in 2004. In 2007, six lynxes (five negative for FeLV in December 2006 and one negative in December 2005, but positive in December 2006) died in a time interval of six months. All six were antigenemic and showed clinical signs and/or hematologic abnormalities, such as anemia, lymphopenia or neutropenia, which are compatible with immunosuppression. Since recent experimental evidence indicates that endogenous retrovirus sequences may mediate post-entry restriction of closely related exogenous retroviruses [1,2], we speculated that endogenous FeLV-related sequences may be underrepresented in the Iberian lynxes with respect to the domestic cat and could therefore have played a role, together with a particular FeLV variant, in defining the high FeLV pathogenicity in the 2007 outbreak.

AIM OF THE STUDY:

- To assess the potential pathogenicity of the FeLV *env* surface unit found in the infected Iberian lynxes and to determine the presence of endogenous FeLV-related sequences (enFeLV) in their genome.

METHODS:

FeLV *env* SURFACE UNIT(SU) CONSENSUS SEQUENCE. TNA from blood or DNA from tissue samples were used for the amplification of the complete FeLV provirus *env* gene. PCR amplification and sequencing primers were based on a multiple sequence alignment (MSA) of all available GenBank FeLV *env* entries and designed to exclude binding to enFeLV and FeLV-C *env* sequences. Sequencing was done directly after PCR amplification and purification of the products.

FeLV-B/FeLV-C SPECIFIC PCR. FeLV subtypes -B and -C *env* specific PCR were performed using primers designed to exclude binding to FeLV-A/C or FeLV-A/B, respectively [3,4].

PHYLOGENETIC ANALYSES. MSA of Iberian lynxes, enFeLV and FeLV subtypes *env* SU proviral DNA translation were built using T-Coffee (http://www.tcoffee.org/). Phylogenetic trees were built using the MEGA3 software. Boostrap support (% of 1,000 bootstrap replicates) was calculated for neighbor-joining, minimum evolution and maximum parsimony methods and considered significant if >70%.

EnFeLV PROVIRAL LOADS. Proviral enFeLV DNA was quantified by Taqman™ real-time PCR using three different systems. Overall, the systems can detect the vast majority of the known enFeLV sequences of the cat genome. To calculate the amount of enFeLV per cell, enFeLV copy numbers were normalized to a fGAPDH pseudogene, of which only one copy is present in the genomic DNA of feline cells [3].

RESULTS:

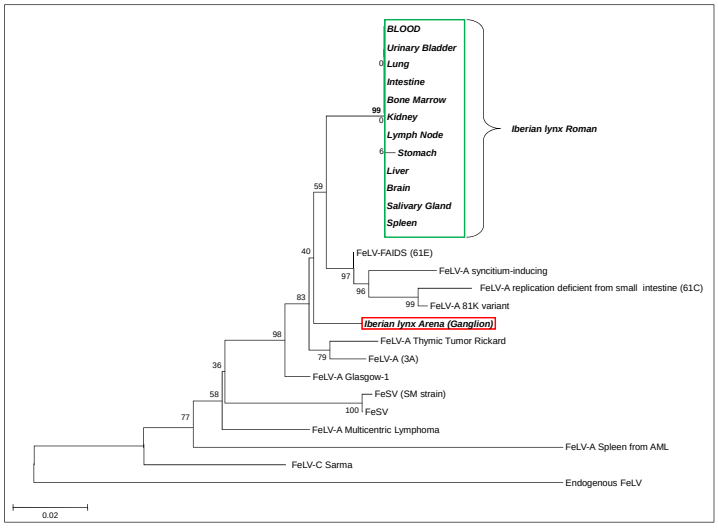

Figure 1. Evolutionary relationships of FeLV envelope SU protein sequences in Iberian lynxes. The Maximum Parsimony (MP) tree is shown. Trees are drawn to scale; length is in the units of the number of changes over the whole sequence. The sequence from lynx "Arena" (the one deceased in 2004) was clearly different from the *env* found in the other ten lynxes, which were nearly identical to each other (maximum 1/432 aminoacid differences at three different sites for three lynxes). Blood and tissues consensuses from Lynx "Roman" are shown as representative sequences. The sequences all clustered together and did not show any of the alterations typical of the immunodeficiency-causing FeLV-A/61C variants.

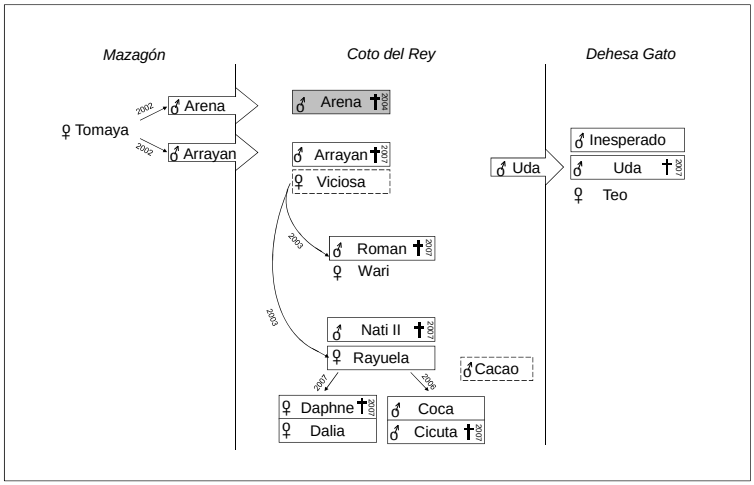

Figure 2. Overview of the geographical distribution and relationships between the FeLV-infected lynxes. The regions of Mazagón, Coto del Rey and Dehesa Gato (Doñana National Park, Spain) are depicted schematically. All animals are identified by names. Large open arrows indicate migration of the specimens after birth, the date of migration is indicated when known. Dead animals are indicated by a cross (†) accompanied by the year of death. Small arrows indicate descendants of the females with birth year. Frames with dashed lines indicate FeLV-infected animals; frames with continuous lines indicate FeLV-infected animals, from whom the FeLV *env* SU were sequenced. Gray shading (animal "Arena") indicates the FeLV variant distantly related to the others (see Figure 1) . The names of lynxes that have been in close contact are positioned close to each other.

EnFeLV and FeLV-B/C subtypes: EnFeLV sequences could be detected only in 10 of the 76 blood samples tested, and at extremely low copy numbers (much less than one copy/cell, a phenomenon that may be explained by a slight crossreactivity of the used real-time PCR systems to not yet identified, FeLV-related genomic sequences). Each of these ten animals was found to be positive only in one out of three different enFeLV assays. FeLV-B and FeLV-C PCR were negative in all FeLV infected lynxes.

CONCLUSIONS:

- The highly pathogenic FeLV infecting the 6 lynxes that succumbed in 2007 and additional 4 living in close contact has the same origin, but potential immunodeficiency-causing mutations are not present in the consensus sequence. The absence of enFeLV from the genome of Iberian lynxes might have played a role in the pathogenicity of this particular FeLV-A variant.

Literature:

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