VETERINARSKI ARHIV 91 (1), 65-71, 2021 DOI: 10.24099/vet.arhiv.0857

A retroviral survey of endangered Eurasian lynx (*Lynx lynx*) from Croatia

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GOMERČIĆ, T., M. PERHARIĆ, J. KUSAK, V. SLIJEPČEVIĆ, V. STAREŠINA, V. STEVANOVIĆ, V. MOJČEC PERKO, I. TOPLIČANEC, M. SINDIČIĆ: A retroviral survey of endangered Eurasian lynx (*Lynx lynx*) from Croatia. Vet. arhiv 91, 65-71, 2021.

ABSTRACT

The feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) may cause persistent, lifelong and lethal infections in domestic and wild felids worldwide. FIV has been confirmed in most *Felidae* species, while FeLV infection is rare among non-domestic cats. The view that retroviruses are pathogenic in domestic cats but not in other free-ranging felid species was disproved by recent findings of retroviral pathology in several wild felids. The epidemiology of retroviral infections in felids in Croatia was only investigated in urban domestic cats, while there are no data for wild cat species. As the reintroduced Dinaric lynx (*Lynx lynx*) population suffers from low genetic diversity, which reduces their ability to adapt to new viral outbreaks, the health status of this lynx population is of particular concern. Two different commercial immunochromatographic assays were used for qualitative detection of FIV antibodies and FeLV antigens, while PCR was used for amplification of proviral *gag* and *env* genes in Eurasian lynx blood samples. All the 17 Eurasian lynx samples collected between 2001 and 2019 tested negative in both immunochromatographic and molecular tests. Even though our sample size was rather small, considering the fact that the population size of lynx in Croatia is estimated at 40 - 60 animals, our results can be considered representative for the population's health status. Also, data about retroviral prevalence in Eurasian lynxes are scarce, so any new findings are very valuable.

Key words: feline immunodeficiency virus; FIV; feline leukemia virus; FeLV; lynx

Introduction

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are significant pathogens of domestic and wild felids worldwide. Both are enveloped RNA viruses, members of the *Retroviridae* family, where FIV is classified to the *Lentivirus* genus, while FeLV belongs to the *Gamaretroviridae*. Due to the enzyme reverse transcriptase and integration of the provirus into

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ISSN 0372-5480
Printed in Croatia

the host cell genome, both viruses cause persistent, lifelong and potentially lethal infections. FIV causes progressive immune dysfunction due to a decrease in $CD4^+$ T-lymphocytes, leading to acquired immunodeficiency syndrome (AIDS). FeLV infection usually causes bone marrow suppression, lymphoma or viral immunosuppression. Retroviral infected domestic cats may have a prolonged period with no clinical signs of the disease, but finally acquire life threatening secondary and opportunistic infections (BULL et al., 2003; HARTMANN, 2011; SELLON and HARTMANN, 2012).

FIV is mainly shed naturally in saliva and is transmitted through bite wounds. Beside bites, transplacental and veneral routes, colostrum and milk are also confirmed modes of FIV transmission (ROGERS and HOOVER, 2002; COATS, 2005; LEVY et al., 2006). FeLV transmission usually occurs through the oronasal route after close contact with salivary secretions, but viremic felids may also shed the virus in nasal secretions, faeces, urine and milk. Vertical transmission of FeLV has also been documented, as well as through cat fleas (PACITTI et al., 1986; VOBIS et al., 2003; GOMES-KELLER et al., 2009).

Both retroviral infections are endemic in domestic cat populations worldwide, with estimated FIV prevalence from 1 to 14% in cats without clinical signs, and up to 44% in cats expressing clinical symptoms (HARTMANN, 1998). The overall prevalence of FeLV in the mixed domestic cat population ranges from 1 to 6% (LEVY et al., 2006; GLEICH et al., 2009; HELLARD et al., 2011). FIV has been confirmed in most of the 37 *Felidae* species (review in O'BRIEN et al., 2012), while FeLV infection is rare among non-domestic cats. Phylogenetic results confirm that although FIV can occasionally move from species to species (TROYER et al., 2008), these events are rare, and most felids carry their own distinct version of the virus (LANGLEY et al., 1994; BARR et al., 1997; PECON-SLATTERY et al., 2008). In contrast, FeLV infections are mostly reported in captive animals that acquired the virus by physical contact with FeLV-infected domestic cats (KENNEDY-STOSKOPF, 1999; CUNNINGHAM et al., 2008).

Three felid species are present in Croatia - the domestic cat (*Felis catus*), the European wild cat (*Felis silvestris silvestris*) and the Eurasian lynx (*Lynx lynx*), but the epidemiological features of retroviral infections have only been investigated in domestic cats from the Zagreb urban area, confirming a high rate of FIV (18.51%) and FeLV (14.5%) seropositive cats (PERHARIĆ et al., 2018). So, the goal of our study was to determine the prevalence of retroviruses in the Eurasian lynx population in Croatia.

The size of the lynx population in Croatia today is estimated at 40 - 60 individuals, all of them being offspring of six individuals reintroduced from Slovakia to the Dinaric Mountains in 1973 (SINDIČIĆ et al. 2016). The health status of the reintroduced Dinaric lynx population is of particular concern, because the population suffers from low genetic diversity and inbreeding depression (SINDIČIĆ et al., 2013). Decreased genetic diversity in wild populations reduces the ability to adapt to new viral outbreaks, such as well-known cases of coronavirus in cheetahs (PEARKS WILKERSON et al., 2004) or different infectious diseses in Iberian lynxes (LÓPEZ et al., 2014).

Materials and methods

The research was performed on 17 wild and captured Eurasian lynx (*Lynx lynx*) blood samples. The animals were sampled in Croatia, in the areas of Gorski kotar and Lika (Fig. 1), in the period from 2001 to 2019. The animals were captured and equipped with radio collars for activity and movement research, except one animal that was found in the vicinity of an urban area, with an injured leg and malnourished. This subadult male lynx died the day after it was captured, with purulent necrotic hepatitis and septicaemia found at necropsy. Apart from this subadult, all the animals were adults, 6 were females and 11 males.

Blood samples were collected from the vena cephalica antebrachi or v. saphena into EDTA-coated tubes by an aseptic method. Retroviral status was initially analyzed by two different commercial immunochromatographic assays - FASTest® FeLV-FIV, MEGACOR Diagnostik GmbH and IDEXX SNAP Combo Plus, IDEXX Laboratories. Both

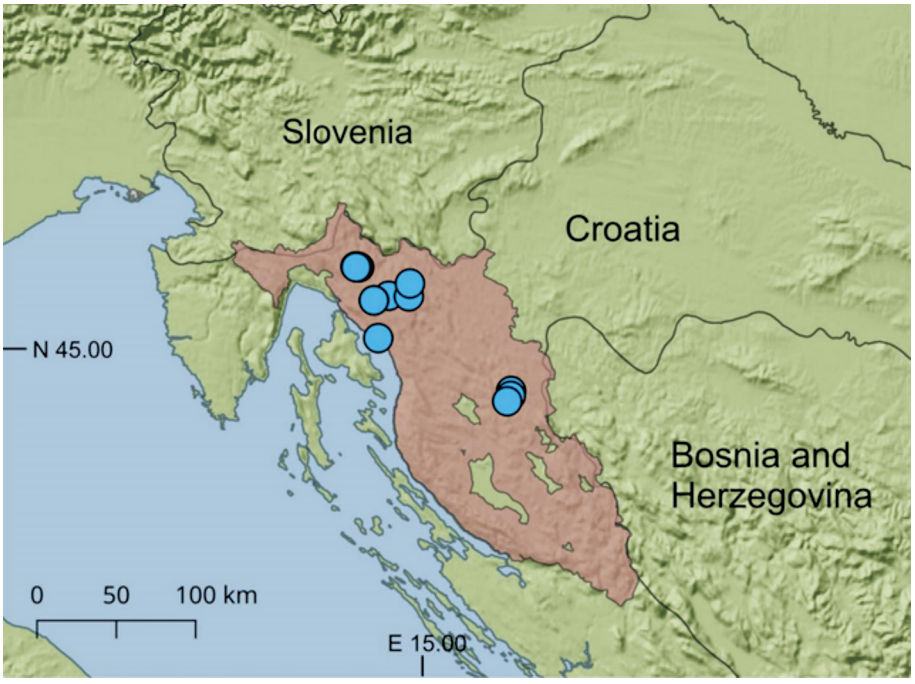

Fig. 1. Map of Croatia, with the brown area indicating Eurasian lynx distribution, and blue dots indicating sampling locations

tests are used for qualitative detection of FIV antibodies for FIV gp40 trans-membrane protein, and qualitative detection of FeLV p27 specific antigens in the whole blood, plasma or serum. The reported sensitivity and specificity for domestic cats for FASTest® FIV were 96.4% and 99.2%, and for FASTest® FeLV 94.7% and 98.8% (HARTMANN et al., 2007). Similar results were published for IDEXX SNAP Combo, sensitivity and specificity for FIV infection were 100% and 99.6%, and for FeLV 92.3% and 97.3% (HARTMANN et al., 2007). Both diagnostic assays were performed according to the manufacturer's instructions.

Prior to molecular diagnostic for FIV and FeLV, DNA was extracted from EDTA-treated lynx whole blood. A commercially available QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used for DNA extraction. Molecular diagnostics for FIV infection were performed with two different PCR protocols. For detection of proviral *gag* gene primers FIV-1026F (5′ - GGC ATA TCC TAT TCA ACC AG - 3′) and FIV 1700R (5′ - AAG AGT TGC ATT TTA TAT CC - 3′) were used for amplification (CAMMAROTA et al., 1996; STEINRIGL and KLEIN, 2003). The second PCR protocol was used for proviral *env* gene detection with primers FIV-7316F (5′ - ATA CCA AAA TGT GGA TGG TG - 3′) and FIV-7868R (5′ - TGC AAG ACC AAT TTC CAG CA - 3′) (STEINRIGL and KLEIN, 2003). For FeLV infection nested PCR was performed for *gag* gene amplification. The first set of primers (outer primers) was U3-F1 (5′ - ACA GCA GAA GTT TCA ACG CC - 3′) and G-R1 (5′ - GAC CAG TGA TCA AGG GTG AG - 3′). The PCR product of this first round served as a template for the second round of PCR with nested primers U3-F2 (5′ - GCT CCC CAG TTG ACC AGA GT - 3′) and G-R2 (5′ - GCT TCG GTA CCA AAC CGA AA - 3′) (KAEWMONGKOL et al., 2007). For both FIV and FeLV, agarose gel electrophoresis was performed to visualize the PCR product.

Results and discussion

All 17 Eurasian lynx tested negative for FIV and FeLV using two different immunochromatographic assays. The FIV proviral *gag* and *env* genes, and the FeLV proviral *gag* gene were not amplified in any of the 17 samples. Even though our sample

size was rather small, considering the fact that the population size of lynx in Croatia is estimated at 40 - 60 animals, our results can be considered representative for the population health status. Also, data about retroviral prevalence in Eurasian lynx are scarce so any new finding is very valuable. The only available data are serological tests for FeLV in free ranging and captive animals from Sweden, and FeLV and FIV in free ranging lynxes from Switzerland, which were all negative (LUTZ et al., 1992; RYSER-DEGIORGIS et al., 2005).

Most data about the retroviral status of lynx in Europe come from Spain, where there is an endangered Iberian lynx (*Lynx pardinus*) population. The absence of FIV antibodies was reported in Iberian lynxes sampled between 1989 and 2007 (ROELKE et al., 2008; LUACES et al., 2008; MILLÁN et al., 2009, MELI et al., 2009), and confirmed by qPCR on 311 individuals sampled between 2004 and 2017 (LOPEZ et al., 2019). Beside negative molecular tests, one animal tested positive for FIV antibodies by ELISA and immunoblot assay, but showed no signs of the disease (LOPEZ et al., 2019). In contrast, an FeLV epidemic caused the death of six Iberian lynxes in 2007, while in the same period an additional seven lynxes were found to be FeLV-positive and survived. Several of the FeLV-infected lynxes showed clinical signs and/or hematological abnormalities, such as anemia, lymphopenia or neutropenia, compatible with the FeLV infection also observed in domestic cats (MELI et al., 2009). As only a few cases of FeLV infection were observed prior to 2007 (LUACES et al., 2008), it is likely that FeLV infection is rare in lynxes, is not readily carried within the lynx population, and most likely originates from domestic cats (MELI et al., 2009). GERET et al. (2011) suggest that the severe outcome of the FeLV outbreak in 2007 was due to the particular susceptibility of the Iberian lynx to pathogens. The Iberain lynx population suffers from reduced genetic variability (JOHNSON et al., 2004), as does our Dinaric lynx population, which underlines the need for regular monitoring of the retroviral status of the population.

The importance of regular monitoring of retroviruses in felids has also been underlined in recent publications. Originally, the absence of clear clinical pathology among infected felids fostered the view that retroviruses are pathogenic in domestic cats but not in other free ranging felid species (CARPENTER and O'BRIEN, 1995; PACKER et. al., 1999; O'BRIEN et al., 2012). However, new findings strongly suggest that FIV is contributing to the loss of immune competence in infected lions (ROELKE et al., 2009), while FeLV was proved as a cause of death in Iberain lynxes in 2007 (MELI et al., 2009), and Florida panthers in 2001-2006 (CUNNINGHAM et al., 2008).

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Received: 28 October 2019
Accepted: 21 May 2020

GOMERČIĆ, T., M. PERHARIĆ, J. KUSAK, V. SLIJEPČEVIĆ, V. STAREŠINA, V. STEVANOVIĆ, V. MOJČEC PERKO, I. TOPLIČANEC, M. SINDIČIĆ: Istraživanje retrovirusnih infekcija u ugroženoj populaciji euroazijskog risa (*Lynx lynx*) u Hrvatskoj. Vet. arhiv 91, 65-71, 2021.

SAŽETAK

Virus mačje imunodeficijencije (FIV) i virus mačje leukemije (FeLV) mogu uzrokovati trajne, cjeloživotne i smrtne infekcije u domaćih i divljih felida širom svijeta. FIV je potvrđen u većine felida, dok je infekcija FeLV-om u domaćih mačaka rijetka. Mišljenje da su retrovirusi patogeni u domaćih mačaka, ali ne i u slobodnoživućih felida, opovrgnuto je najnovijim istraživanjima retrovirusne patologije u nekoliko divljih felida. Epidemiologija retrovirusnih infekcija u felida u Hrvatskoj istraživana je u gradskih domaćih mačaka, dok podaci za divlje mačke ne postoje. Kako populaciju reintroduciranog dinarskog risa (*Lynx lynx*) obilježava niska genetska raznolikost, što smanjuje mogućnost prilagodbe na nove virusne zaraze, postoji osobita zabrinutost za zdravstveno stanje ove populacije risa. U radu su upotrijebljene dvije različite komercijalne imunokromatografske pretrage za kvalitativnu detekciju protutijela na FIV i FeLV antigene, dok je PCR upotrijebljen za umnažanje provirusnih gena *gag* i *env* u uzorcima krvi euroazijskog risa. Svih 17 uzoraka euroazijskog risa, prikupljenih od 2001. do 2019., bilo je negativno i u imunokromatografskom i molekularnom testu. Iako se radi o maloj veličini uzorka, s obzirom na to da je veličina populacije risa u Hrvatskoj procijenjena na 40 – 60 jedinki, naši se uzorci mogu smatrati reprezentativnima za zdravstveni status navedene populacije. Također, malo je podataka o retrovirusnoj prevalenciji u euroazijskog risa, što novim podacima dobivenim ovim istraživanjem daje dodatnu vrijednost.

Ključne riječi: virus mačje imunodeficijencije; FIV; virus mačje leukemije; FeLV; ris