Theses of a doctoral (PhD) dissertation

EXAMINATION OF HUNGARIAN PREVALENCE OF FELINE RETROVIRUSES WITH MOLECULAR BIOLOGICAL METHODS AND GENETIC CHARACTERIZATION OF SOME OF THESE VIRUSES

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Table of contents

1. Aims of the study

Until recently, only data of minor, local studies or serological researches conducted 30 years ago were available in Hungary related to retroviral prevalence in domestic cats. Primarily we would like to address this shortcoming with this PhD-study.

The aims of the study were:

Ad 1. to make a comprehensive epidemiological study on domestic cat blood samples, using two methods (ELISA and PCR) to ensure data accuracy, and to determine the prevalence of feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) in Hungary.

Ad 2. to analyse partial polymerase (*pol*) gene sequences of obtained FIV strains and to make a filogenetic study of these strains determining the origin and relation of them.

Ad 3. to develop nem methods, such as RNAscope *in situ* hybridization, in order to detect viral RNA in feline tissues and FeLV/FIV-related lesions.

Ad 4. to continue our study in the Republic of Ireland, and to make the same research on collected Irish blood samples, using the same methods as in Hungary.

2. Summary

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are retroviruses causing various diseases in domestic and wild felid populations worldwide. The objectives of the study were to estimate the prevalence of these retroviruses in domestic cats in Hungary and to characterise the phylogenetic relationships of FIV strains.

A total of 335 anticoagulated whole-blood samples obtained from both healthy and ill cats from all over Hungary were examined for the presence of FIV and FeLV between 2016 and 2018. We excluded all shelter and stray cats from study. Two methods were used for the diagnostics: point-of-care ELISA directly after blood drawing, followed by PCR at the University of Veterinary Medicine Budapest, Department of Pathology. The data obtained were analyzed and compared by different statistical assays. True prevalence calculated from the ELISA results was 9.89% and 11.78% for FIV and FeLV, respectively. Apparent prevalence obtained from the PCR results was 13.13% for FIV and 17.31% for FeLV. Sequencing and phylogenetic analysis of partial polymerase (pol) gene of 22 FIV strains showed that they all belonged to FIV subtype B. The strains were grouped into several monophyletic subgroups reflecting the geographic locations of the origin of the samples. The overall mean genetic similarity between the strains was 98.2%.

Moreover, we had the opportunity to broaden the spectrum of our study and make a similar research in the Republic of Ireland, between 2017–2018. We collected a total of 183 anticoagulated whole-blood samples. Conditions and examination methods were the same of the Hungarian part of study. Apparent prevalence calculated from ELISA results was 10.87% for FIV and 3.28% for FeLV, and true prevalence was 4,9% for FIV (true prevalence could not be obtained in case of FeLV). Apparent prevalence obtained from PCR results was 9,3% and 11.63% for FIV and FeLV, respectively. Phylogenetic analysis of partial pol gene sequences of 8 FIV strains showed that 7 belonged to subtype A and 1 belonged to subtype B.

We have also developed an RNA based in situ hybridization assay (RNAscope) to detect the presence of FIV and FeLV viral nucleic acid in feline tissues obtained during pathological investigations at our Department. We focused on the cases, where retroviral infection has been confirmed previously or we observed lesions suggesting the infection during necropsies. We have successfully visualized the viruses in lymphoid, bone marrow and other tissues as well as in several specific lesions caused by FIV and/or FeLV infection. We detected FeLV RNA in a rare, extranodal, primary lymphoma of the spinal cord, after clinicopathologic evaluation and

immunophenotyping. Moreover, FeLV-infection and presence of the virus within lesions were confirmed in the background of a rare case of feline osteochondromatosis.

We have reported the first thorough, systematic overview on the prevalence of FeLV and FIV in both Hungary and Ireland and gave insight into the genetic diversity of FIV.

3. New scientific results

1. We made the first comprehensive and representative, large-scale prevalence study of FeLV and FIV in Hungary and Ireland with two examination methods (ELISA and PCR).

2. We have determined the partial genetic sequence of obtained Hungarian and Irish FIV strains, and made a comparative filogenetic study on them. In Hungary, mostly FIV subtype B is prevalent, on the other hand in Ireland, subtype A is prevalent.

3. We developed an RNAscope *in situ* hybridization method for specific demonstration of feline retroviruses, and we demonstrated a case of feline osteochondromatosis in Hungary first, detecting FeLV in parenchymatous organs and in neoplastic lesions with *in situ* hybridization also.

4. Publications

- Szilasi A., Balka Gy.: **A macskák retrovírus fertőzései: Feline Immunodeficiency Virus (FIV) Irodalmi áttekintés 1. rész,** Magy. Allatorvosok Lapja, 137. 351-360, 2015.

- Szilasi A., Reinhard, E., Balka Gy.: **A macskák retrovírus fertőzései: Feline Immunodeficiency Virus (FIV) Irodalmi áttekintés 2. rész**, Magy. Allatorvosok Lapja, 137. 729-738, 2015.

- Szilasi, A., Dénes, L., Balka, Gy.: **A macskák retrovírus fertőzései: Feline Leukemia Virus (FeLV) Irodalmi áttekintés**, Magy. Allatorvosok Lapja, 140. 457-472, 2018.

- Szilasi, A., Dénes, L., Krikó, E., Heenemann, K., Ertl, R., Mándoki, M., Vahlenkamp T. W., Balka, Gy.: **Prevalence of feline immunodeficiency virus and feline leukaemia virus in domestic cats in Hungary**, J. Feline Med. Surg. Open Reports, 5(2). 1-7, 2019.

- Szilasi, A., Dénes, L., Jakab, Cs., Erdélyi, I., Resende, T., Vannucci, F., Csomor, J., Mándoki, M., Balka, Gy.: **In situ hybridization of feline leukemia virus in a primary neural B-cell lymphoma**, J. Vet. Diagn., 32(3). 454-457, 2020.

- Szilasi, A., Dénes, L., Krikó, E., Murray, C., Mándoki, M., Balka, Gy.: **Prevalence of feline leukaemia virus and feline immunodeficiency virus in domestic cats in Ireland**, Acta Vet. Hung., 68(4). 413-420, 2020.