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ORIGINAL RESEARCH PAPER

Prevalence of feline leukaemia virus and feline immunodeficiency virus in domestic cats in Ireland

ANNA SZILASI[1*], LILLA DÉNES[1], ESZTER KRIKÓ[2], CAOIMHE MURRAY[3], MÍRA MÁNDOKI[1] and GYULA BALKA[1]

[1] Department of Pathology, University of Veterinary Medicine, István u. 2, H-1078 Budapest, Hungary

[2] Centre for Bioinformatics, University of Veterinary Medicine, Budapest, Hungary

[3] Suirside Veterinary Clinic, Townparks, Carrick-On-Suir, Co. Tipperary, Ireland

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ABSTRACT

Feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) are retroviruses affecting felid species worldwide. A study was performed over a period of 5 months in Ireland with the aim to get an updated and more realistic prevalence of these retroviruses. A total of 183 EDTA-anticoagulated whole-blood samples were collected from cats distributed between 10 clinics. The samples were tested using both point-of-care enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Basic clinical data and vaccination history were also recorded for the sampled cats. The results of ELISA tests showed a prevalence of 10.4 and 3.3% for FIV and FeLV, respectively, and an apparent prevalence of 9.3% for FIV and 11.6% for FeLV with PCR. Phylogenetic analysis of the partial polymerase (*pol*) gene sequences obtained from 8 FIV-positive strains showed that all but one of the Irish strains belonged to FIV subtype A, and one to subtype B. The overall mean genetic similarity between the analysed strains was 91.15%.

KEYWORDS

feline, retrovirus, prevalence, phylogeny, Ireland

INTRODUCTION

Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are enveloped single-stranded RNA viruses belonging to the *Retroviridae* family. These viruses are a threat to the feline species all over the world; however, effective vaccination is only available against FeLV infection. Formerly, FeLV was the most important viral pathogen among domestic cats (*Felis catus*) (Dunham and Graham, 2008). Nowadays, due to extensive screenings and the use of vaccination protocols, this has changed. The current vaccine against FIV is only commercially available in a few countries and is not efficient enough due to the high variability of the virus. FIV is the cause of an acquired immune deficiency syndrome (AIDS) in cats, resembling the same syndrome caused by human immunodeficiency virus (HIV) in humans (Bienzle, 2014). Therefore, it is vitally important to gain more knowledge on this virus as it is a model for prophylactic and therapeutic studies of HIV.

According to phylogenetic analyses, FIV strains can be grouped into seven subtypes (A–F, U-NZenv), and these clades show up to cc. 26% genetic differences among their envelope (*env*) gene sequences (Steinrigl and Klein, 2004; Duarte and Tavares, 2006; Hayward and Rodrigo, 2008). The subtypes are differentiated by the genetic sequence of the V3–V5 region of the *env* gene. The occurrence of various subtypes is geographically different. Some

*Corresponding author. Tel.: +36 20 490 2128.
E-mail: szilasi.anna@univet.hu

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subtypes, such as A, B and C, are more widely spread than others. The distribution of the reported subtypes is illustrated on Fig. 1.

Subtype B represents an older evolutionary cluster and its members seem to be less pathogenic than subtype A strains (Beczkowski et al., 2015). Co-infections caused by multiple subtypes may lead to the so-called 'superinfection', which results in higher virulence and pathogenicity due to recombination events of the viral genomes (Kann et al., 2006; Beczkowski et al., 2015).

To test a cat's infection status, point-of-care enzyme-linked immunosorbent assay (ELISA) snap tests are widely used, which detect the p27 antigen of FeLV and antibodies against the p24 protein of FIV. The American Association of Feline Practitioners (AAFP) prepared a guideline in 2008 (and updated it in 2020) about the identification and management of retroviral diseases, which contains the recommendations for screening and confirmatory test possibilities (Little et al., 2020). The most frequent test used to confirm ELISA results or in the case of false/non-interpretable results is polymerase chain reaction (PCR).

Previous studies have reported a relatively low prevalence of these retroviruses worldwide. In North America, FeLV prevalence ranges between 2.3–7.5 and 2% in Australia, while in Europe it is somewhat higher, 3.6–15.6%. FIV prevalence levels are almost similar: 2.5–7.5% in North America, 15% in Australia, and 3.2–8.3% in Europe (Studer et al., 2019; Little et al., 2020). In a similar study, our research group showed a true prevalence of 11.8% for FeLV and 9.9% for FIV with ELISA. Apparent prevalence values calculated from the PCR results were 17.3% for FeLV and 13.1% for FIV in Hungary (Szilasi et al., 2019).

So far, only minor studies have been carried out in Ireland with a relatively small number of samples. In 2011, 112 blood samples were tested for the presence of FIV antibodies and FeLV antigens during a study on feline blood transfusions. These samples were obtained from the Dublin area, and the prevalence for the viruses FIV and FeLV was 4.35 and 1.09%, respectively (Juvet et al., 2011). Major studies were carried out in the United Kingdom (UK): in 1989, 2,211 blood samples were tested with a prevalence of FIV 19% in sick cats, and 6% in healthy ones. For FeLV, the prevalence was 18% in sick, and 5% in healthy individuals (Hosie et al., 1989). In 2002, 517 stray cats admitted to an RSPCA veterinary hospital were tested with a result of 10.4 and 3.5% for FIV and FeLV, respectively (Muirden, 2002).

Only limited information is available in the literature on phylogenetic analyses. One study carried out in the UK in 2011 revealed FIV subtype A infection with the examination of 47 FIV strains obtained from naturally infected domestic cats, covering the full spectrum of subtype A genetic diversity (1 identified sequence was likely a subtype A/C recombinant) (Samman et al., 2011). These prevalence and phylogenetic data cover Northern Ireland, but not the Republic of Ireland (except the study made in the Dublin area; however, as the samples were taken from cats living in a small region of the country, these values cannot be extrapolated to the whole country). Thus, the aim of our study was to get an updated and more representative prevalence of these retroviruses in Ireland, and to gain knowledge on FIV phylogenetics.

MATERIALS AND METHODS

A total of 183 randomly selected, client-owned domestic cats were tested from 10 veterinary clinics in Ireland (one in Dublin, Wicklow, Kilkenny, Sligo, Galway, two in Cork and three in Wexford). Localisation of the regions sampled is shown in Fig. 2. The sampling period was from August 2017 to January 2018. No sheltered or free-roaming cats were included in the survey. Age, sex, vaccination status and house holding status of the cats were registered. Patient history was also taken from each cat looking for any clinical sign in its earlier life and at time of the sampling. General physical examination was completed in each case and EDTA-anticoagulated blood samples were collected as a part of the routine diagnostic or screening process. The samplings were carried out according to the latest ethical and animal welfare guidelines and regulations of Ireland; the cats were originally subjected to blood analysis for other reasons. (No extra handling and puncturing were carried out only for

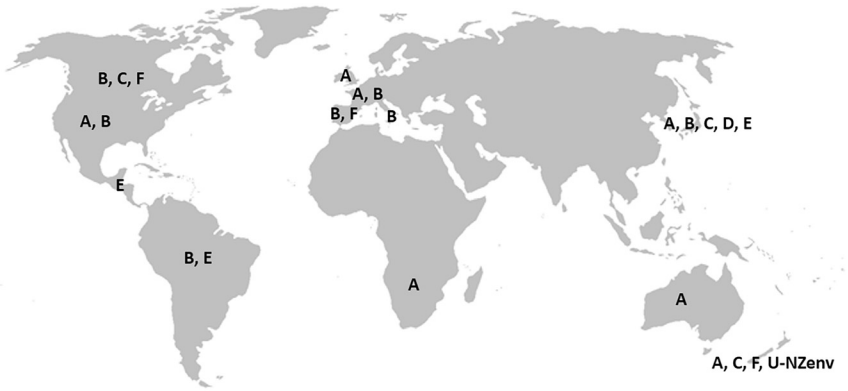

Fig. 1. Distribution of feline immunodeficiency virus subtypes around the world (map scheme: www.outline-world-map.com) (Szilasi et al., 2019). FIV subtypes A and B are spread widely, whereas subtypes C, D, E, F and U-NZenv are distributed only regionally

Fig. 2. A representation of the areas of Ireland covered in this research, marked with red pinpoints [map scheme: https://www.familytreemagazine.com/premium/irish-counties-map/#]

the sake of the study.) Immediately after the blood was drawn, Witness FeLV-FIV ELISA test (Zoetis) was performed according to the manufacturer's instructions. The rest of the samples were frozen and sent to the Department of Pathology, University of Veterinary Medicine Budapest, and were stored at –80 °C until further examination.

Serology testing

The test used in this study is an accurate and simple point-of-care test that detects the presence of FeLV p27 antigen (92.9% sensitivity, 96.5% specificity) and FIV antibodies against the p24 antigen (93.8% sensitivity, 93.4% specificity) (Zoetis Inc. D880Z-US-14-013; D880Z-US-14-014). Sensitivity and specificity values were previously estimated by the manufacturer, compared to the gold standard methods. The test is based on rapid immunomigration. Sensitivity and specificity values used in the statistical analyses were given according to the data provided by Zoetis, although vaguely different values can be found in some field studies (e.g. 89.0/95.5% for FeLV and 94.7/100% for FIV) (Westman et al., 2019). One drop (0.05 mL) of EDTA-anticoagulated whole-blood was used according to the manufacturer's instructions.

Polymerase chain reaction

Nucleic acid extraction from the stored whole-blood specimens was carried out using a QIAcube instrument (Qiagen, Hilden, Germany) with the QIAmp cador Pathogen Mini Kit (Qiagen), according to the manufacturer's instructions. Nucleic acid was eluted in 60 µL RNase-free distilled water (Qiagen). The preparation of end-point PCR for FIV, Top-Taq Master Mix Kit (Qiagen) was used in accordance with the manufacturer's instructions: 25 µL master mix (final concentrations: 1 × of Buffer; 200 µM of each dNTP; 1.25 units of DNA polymerase/reaction), 0.5 µL forward and reverse primers each (40 µM; final concentration: 0.2 µM), 5 µL CoralLoad Concentrate (final concentration: 1×), 18 µL RNase-free water, and 1 µL template DNA were mixed together for each sample. Genesy PCR Thermal Cycler (Tianlong) was used for hot-start PCR amplification with the given protocol: 95 °C for 15 min in stage 1; 95 °C for 45 sec and 60 °C for 45 sec and 72 °C for 1 min in stage 2 (40 cycles); 72 °C for 15 min in stage 3. The primers used to detect FIV were: LTR-sense 5′-GCGCTAGCAGCTGCC-TAACCGCAAAACCAC-3′ and LTR-antisense 5′-GTATCTGTGGGAGCCTCAAGGGAGAACTC-3′ (Sutton, 2007). We used One Step RT-PCR Kit (Qiagen) for the detection of FeLV, according to the instructions in the user manual. Master mix containing 5.7 µL Rnase-free water, 2 µL 5 × buffer (final concentration: 1×), 0.4 µL dNTP Mix (final concentration: 400 µM of each dNTP), 0.4 µL enzyme mix (no concentration is given by the manufacturer), 0.1 µL RNase inhibitor (final concentration: 5 units/reaction), 0.2 µL forward and reverse primers each (40 µM; final concentration: 0.6 µM), was added into tubes with 0.5 µL sample. Thermal conditions were: 50 °C for 30 min and 94 °C for 3 min in the stage of reverse transcription and initial denaturation; 95 °C for 15 sec and 60 °C for 1 min and 72 °C for 1 min in stage 2 (45 cycles); 72 °C for 10 min. FeLV primers were obtained from Tandon et al. (2005): LTR-sense 5′-AACAGCAGAAGTTTCAAGGCC-3′ and LTR-antisense 5′-TTATAGCAGAAAGCGCGCG-3′. All primers amplify the early-stage products of reverse transcription. The amplicons were 163 base pair (bp) (FIV) and 150 bp (FeLV) long. PCR products were visualised by electrophoresis in 1.5% agarose gel.

Statistical analysis

The statistical calculations were performed in an R environment (R Core Team, Austria) and a logistic regression model was used to detect the relationship between infection status and examined variants (R Core Team, 2016). The epi.prev function was used to calculate prevalences (Sullivan et al., 2009). The value of Cohen's kappa was estimated to show possible cross-compliance between ELISA and PCR methods (Dinya and Solymosi, 2016).

Phylogenetic analysis

In the case of FIV PCR-positive cases, further sequencing was carried out to gain insight into the genetic diversity of Irish FIV strains. Partial proviral *pol* genes were amplified using the previously described protocol of Adams et al. (2010). All primers were synthesised by Sigma Genosys (The Woodlands, TX, USA) and reconstituted to a 20 pM

working solution. PCR products were subjected to gel electrophoresis, and the amplicons of 576 bp length were cut out manually and purified by the use of Qiagen Gel Extraction Kit (Qiagen). Bidirectional Sanger sequencing reaction was performed with the corresponding primers, and capillary electrophoresis was made by a commercial provider (Hungarian Natural History Museum, Hungary). GenBank accession numbers are MT782321–MT782328.

After proofreading of the sequences obtained, they were assembled using the E–INS–I method of the online software Mafft version 7 (Katoh and Toh, 2008) and aligned against available FIV genomes downloaded from GenBank that represent the overall diversity of the virus. Maximum Likelihood analyses were carried out and the trees were visualised and edited with MEGA7 (Kumar et al., 2016) using the Tamura-Nei model with 1,000 bootstrap replicates and gamma distribution (Tamura et al., 2013). Pairwise genetic analyses were conducted using the Kimura 2-parameter model (Kimura, 1980) in MEGA7.

RESULTS

In total, 183 samples were included in the study. All were client-owned domestic cats, no stray or shelter animals were included. The sampled animals were characterised by age, sex, clinical signs, and immunisation (Table 1). The mean age of the cats was 4.85 years in this study (ranging from 3 months to 21 years). In summary, a total of 88 females (48.09%) and 95 males (51.91%) were included. Out of the 88 females, 32 (36.36%) were intact and 56 (63.64%) were spayed; of the 95 males, 38 (40.00%) were intact and 57 (60.00%) were neutered. Forty cats (21.86%) were kept strictly indoors and 143 (78.14%) had outdoor access. A total of 85 cats (46.45%) were clinically healthy during the physical examination. Only 63 (34.43%) cats were immunised at some point of lifespan, usually with a combined vaccine (an overall low prevalence of vaccination), and among these cats, only 45 (24.6%) were vaccinated against FeLV too. Most of the FeLV vaccinations were adequate and up-to-date; however, we found two PCR-positive cats (one of these cats' vaccination was out-of-date) and one ELISA-positive cat among these.

Out of the 183 samples, 6 (3.28%) and 19 (10.38%) were positive with the Witness ELISA test kit for FeLV and FIV, respectively. According to the PCR tests, however, 20 (10.93%) were positive for FeLV and 16 (8.74%) for FIV. FeLV–FIV co-infection was identified in 1 case (0.55%). In some cases, only one test method was positive: in the case of FIV, 9/19 ELISA tests and 6/16 PCR test results were positive, when the parallel assay was negative. In the case of FeLV, 2/6 ELISA results and 16/20 PCR results were single-positive (Table 2).

Total apparent prevalence, according to the Witness test, was 3.28 (95% CI: 1.51–6.97) in the case of FeLV and 10.87 (95% CI: 7.15–16.19) in the case of FIV. True prevalence was also calculated with the previously estimated sensitivity and specificity of the ELISA test: 4.90 (95% CI: 0.63–11.00) for FIV. In the case of FeLV, the true prevalence could not be calculated.

Apparent prevalence according to the PCR results was 11.63 (95% CI: 7.65–17.28) and 9.30 (95% CI: 5.81–14.58) for FeLV and FIV, respectively. Cross-compliance was analysed by calculating the value of Cohen's kappa coefficient. This coefficient was 0.287 (95% CI: 0.08–0.55) for FeLV (with a P value <0.05) and 0.523 (95% CI: 0.30–0.73) for FIV ($P < 0.0001$). Both indicate a significant coherence between the Witness ELISA and the PCR results. A summary of these results is shown in Table 3.

We performed logistic regression, searching for relationships between some variants (age, sex and keeping

Table 1. Summary of basic data of cats sampled in the study. Age, sex, vaccination status and house holding status were registered. Patient history was taken from each cat looking for any clinical sign in its earlier life and at time of the sampling (healthy/unhealthy status)

	Female (n, %)	Male (n, %)	Total number (n, %)
Sex	88 (48.09%)	95 (51.91%)	183 (100%)
Intact	32 (36.36%)	38 (40.00%)	70 (38.25%)
Spayed/neutered	56 (63.64%)	57 (60.00%)	113 (61.75%)
Indoor	19 (10.38%)	21 (11.48%)	40 (21.86%)
Outdoor	69 (37.70%)	74 (40.44%)	143 (78.14%)
Healthy	42 (22.95%)	43 (23.50%)	85 (46.45%)
Unhealthy	46 (25.14%)	52 (28.42%)	98 (53.55%)
Vaccinated (combined)	41 (22.40%)	22 (12.02%)	63 (34.43%)
Vaccinated (FeLV)	26 (14.21%)	19 (10.38%)	45 (24.6%)

Table 2. Summary of positive test results reported in the study. Total numbers and percentages of retrovirus-positive samples with ELISA and PCR tests, from the 183 blood specimens are shown. Numbers in brackets reveal those samples, which were positive only with one test method

	FIV	FeLV
ELISA	19 (9) 10.38%	6 (2) 3.28%
PCR	16 (6) 8.74%	20 (16) 10.93%

Table 3. Summary of prevalence data reported in the study. Apparent prevalences are shown according to Witness ELISA and PCR tests. True prevalence could be calculated with the previously estimated sensitivity and specificity of the ELISA test

	FIV ELISA	FIV PCR	FeLV ELISA	FeLV PCR
Apparent prevalence	10.87%	9.30%	3.28%	11.63%
True prevalence	4.90%	–[a]	–[b]	–[a]

[a]Could not be estimated in the lack of sensitivity and specificity values of the PCR method used.
[b]Could not be estimated (1 – $\text{specificity}_{\text{Witness}}$ < apparent prevalence).

conditions) and retroviral infection status. A higher chance of infection with FeLV was found in correlation with outdoor access (Pearson's residual 0.61, $P = 0.342$), and a higher chance to be infected with FIV (Pearson's residual 0.82, $P = 0.08$), both counted from results with the ELISA method. Calculating with the PCR results, $P = 0.08$ for FeLV (Pearson's residual 0.8) and $P = 0.28$ for FIV (Pearson's residual 0.66) were counted in relation to outdoor access. These data show the tendencies that are also confirmed by other studies, although the values are not significant in our case.

No significant correlation was found with the animals' age regarding retroviral infections (either with results of ELISA or PCR). We found a correlation between infection status and sex: males were more likely to get FIV-infected than females (Witness FIV $P = 0.15$; PCR FIV $P = 0.19$). In the case of FeLV infection, ELISA results showed a higher chance of positivity in males ($P = 0.68$), although it was not significant. FeLV PCR results do not show correlation between sex and infection status.

We were able to sequence partial *pol* genes in the case of 8 samples out of the 16 FIV PCR-positive cases. We suspect that the rest of the samples contained insufficient amounts of, or poor-quality, nucleic acids. Multiple alignments and Maximum Likelihood tree reconstructions prepared from the Irish sequences and a backbone set of available sequences representing overall FIV genetic diversity revealed that all but one of the strains were clustered into subtype A, whereas one belonged to subtype B, as shown in Fig. 3. Within clade A, the Irish sequences were grouped into a monophyletic group. The strain in clade B seems to be related (genetic similarity 93.7%) to a strain originating from Joinville, Brazil, 2017 (acc. number: KY629414). The subgroups within clade A are supported by relatively high bootstrap values.

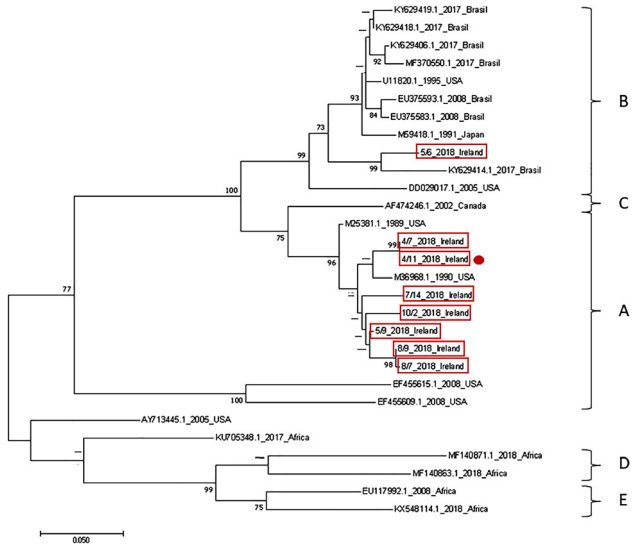

Fig. 3. Phylogenetic tree reconstructed from the partial *pol* gene sequences of Irish feline immunodeficiency virus strains and selected reference sequences from GenBank. Maximum likelihood bootstrap support values are shown as percentages above the branches. The indoor cat is marked with red dot; all the others had outdoor access. In case of reference sequences obtained from GenBank, accession number, year of publication and country of collection are displayed. Irish sequences are framed with red

Pairwise genetic analyses revealed that the overall mean genetic similarity between the strains included in our study was 91.15% (lowest similarity was 82.48% and the highest was 99.81%). All but one cat with sequenced virus DNA had outdoor access (7 outdoor, 1 indoor), and most of the cats were males (6 males, 2 females).

DISCUSSION

Both the apparent prevalence counted from both ELISA (10.87%) and PCR (9.30%) results and the true prevalence from ELISA (4.90%) results show a relatively high rate of infection in the case of FIV in owned domestic cats in Ireland compared to the most recent data from surrounding (or more distant) regions and countries – 4.35% in Dublin; 19% (sick cats) and 6% (healthy cats) in the UK; 3.2–8.3% in Europe – (with the exclusion of prevalence data including free-roaming or shelter cats). The prevalence rate of FeLV does not show such a difference (Hosie et al., 1989; Juvet et al., 2011; Studer et al., 2019). In the background of the lower prevalence rates of FeLV infection, we can speculate the wide and already long-term vaccination practice against the virus and the constant screening for retroviral infections. The use of the vaccine is a highly effective tool to prevent the infection (Lutz et al., 2009). Data regarding the vaccination status collected along with the sampling of the cats, showed that there is still a need for improvement in the vaccination prevalence in Irish cat populations, as among the cats included in the study, 24.6% were vaccinated against FeLV. Two of these immunised cats had FeLV-positive results with the PCR method (and one had an FeLV-positive ELISA result without the proof of PCR positivity), but their vaccinations were not regular or up-to-date, which could have led to unprotected and susceptible periods (Hofmann-Lehmann et al., 2014). Moreover, 78.14% of the cats had outdoor access, and most of them were intact, which means a higher risk for infection due to more aggressive behaviour (Goldkamp et al., 2008).

With regard to FIV, currently no commercial vaccine is available in Ireland; therefore, there is no discrepancy in the interpretation of ELISA results, although at present we can already distinguish between vaccine and field strains of FIV (Westman et al., 2015, 2016). According to studies, two point-of-care FIV-antibody tests (Witness FeLV/FIV and Anigen Rapid FIV/FeLV) were able to correctly identify FIV infection status irrespective of whether or not the cats were vaccinated. In case of doubtful results, we still have the opportunity to perform a confirmatory PCR test.

There is also another possible explanation for the higher prevalence data, which could be the weak positive predictive value of every diagnostic test that does not have 100% specificity in the case of low prevalence rates in a population. In that case, the possibility of a false positive reaction is

higher than the chance of a true positive reaction (false positive paradox). However, using a second confirmatory diagnostic assay (PCR), the predictive value can be improved, and the final result will be more reliable.

The discrepancies observed between ELISA and PCR results, where only one test method was positive, can be explained by multiple causes. In the case of FIV, the Witness test detects antibodies, meanwhile the PCR amplifies and detects part of the long terminal repeat region of the virus. In the early stages of infection, there are no detectable antibodies (most cats produce antibodies 8 weeks after the exposure) (Sykes and Greene, 2012). In addition, there might be extremely low levels of antibodies in the terminal phase of the disease, causing false negative ELISA results, whereas the PCR will be positive due to the increased viral load in circulation (Westman et al., 2019). On the other hand, positive ELISA and negative PCR results can suggest low levels of circulating proviral DNA/viral RNA (which can be the case in the asymptomatic phase of FIV infection) (Westman et al., 2019). The presence and interference of maternal antibodies with the ELISA test can be excluded in our cases as the youngest animals included in the study were FIV negative. Only one 5-month-old kitten was FIV positive with the ELISA method, but the infection in that case was confirmed by PCR as well. Maternal antibodies can be present in kittens up to 6 months of age (MacDonald et al., 2004). In the case of FeLV, the Witness test detects the p27 antigen, whereas the PCR amplifies and detects the U3 part of the LTR region. Negative ELISA and positive PCR results can also suggest an early phase of infection or illness in a regressive stage, when small amounts of proviral DNA/viral RNA are already present in the circulation, but the amount of the antigen is still below the detectable levels.

According to the phylogenetic analyses and tree reconstructions of the FIV sequences, all but one sequence belonged to subtype A, which is in harmony with previous publications from the UK (Samman et al., 2011; Studer et al., 2019). The monophyletic pattern suggests a possible common ancestor for most of the Irish sequences analysed in the study. Based on our data, it can be speculated that there were at least two events of virus introduction into the country, but only subtype A was able to spread among domestic cats and its genetic diversity is the result of divergent local evolution of the strains. The presence of the subtype B sequence (in close relation to a Brazilian sequence – genetic similarity 93.7%) might suggest an epidemiological relation between cats of Ireland (or at least this animal) and Brazil, e.g. cats imported or travelling from one country to the other. (According to the Irish regulations, cats entering the country from abroad must only be vaccinated against rabies.) The import of infected semen can also be suspected; however, our questionnaire did not include travel data and information regarding reproduction. Interestingly, the same Brazilian sequence was also related to Hungarian FIV strains sequenced by our group in 2018 (Szilasi et al., 2019), which could suggest that this strain is, or more likely is related to, a common ancestor that later spread.

Some of the closest pairs of strains forming monophyletic subgroups originate from animals within the same geographic region, and all cats but one had outdoor access. This suggests that infected cats with outdoor access can shed and transmit the virus locally, and genetically similar strains circulate among cats in each geographic area. Closely related strains from different locations might suggest travelling of the owners along with their pets, which is getting more and more popular nowadays. The evidence of local circulation of FIV strains emphasises the importance of neutering and effective preventive actions (such as vaccination in the case of FeLV); however, we did not find significant differences between the prevalence of intact and sterilised populations, although the tendency of higher infection rate of intact males can be seen (Addie et al., 2000).

Although it must be highlighted that the 8 sequences we were able to sequence are insufficient to give a complete depiction of the circulating FIV strains in Ireland, it can be concluded that mainly subtype A strains are present in the country. This result correlates well with data published from surrounding countries, where mostly subtype A was discovered, but we should also emphasise the possible presence of other subtypes, such as subtype B. This dataset and the constant, prospective phylogenetic analyses of further FIV-positive strains can give useful information for vaccine developers. The presence of subtypes other than subtype A (or D) raises the question of whether the only existing FIV vaccine, which is not available now in Ireland (Fel-O-Vax FIV, Boehringer Ingelheim), would be efficient, as the vaccine contains inactivated dual-subtype (subtypes A and D) FIV-infected cells, that will not confer sufficient protective effect against subtype B or other subtypes (Yamamoto et al., 2002; Kann et al., 2006; Sahay and Yamamoto, 2018).

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