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## Prevalence of feline coronavirus, feline leukemia virus, and feline immunodeficiency virus in client-owned cats in Croatia

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### Abstract

This study aimed to determine prevalences for anti-FCoV antibody, FeLV antigen, FeLV proviral DNA, and anti-FIV antibody among client-owned cats from the cities of Zagreb and Varaždin in Croatia. Subjects included 106 client-owned cats tested at the Faculty of Veterinary Medicine, Vienna, Austria. Blood samples were tested with IFA for anti-FCoV antibody and IFA FCoV antibody titers, with ELISA for FeLV p27 antigen, with PCR for FeLV proviral DNA, and with RIM for anti-FIV antibody. Prevalence of FCoV and FeLV was 41.51% and 6.60%, respectively. A coinfection with FeLV/FCoV and FIV/FCoV prevalence was 7.55% and 5.66%. No cats were coinfecting with FIV and FeLV. All three viruses were detected, confirming their presence in Croatia. The seroepidemiological findings demonstrate that both feline retroviruses and feline coronavirus are important feline pathogens in Croatia.

**Keywords:** FCoV, FeLV, FIV, cat, Croatia

### Introduction

Feline coronavirus (FCoV) belongs to the family of *Coronaviridae* within the order *Nidovirales* [1,2,3]. Infection with FCoV is prevalent in pet cats [4]. The prevalence of FCoV in the cat population is very high in multi-cat environments [4]. For example, one study in Israel, Baneth et al., 1999 [5] reported that 83.3% of cats from animal shelters were positive for antibodies against FCoV. In Malaysia, the positive rate of FCoV infection was 84% in cats from catteries [4]. European prevalence is reported to range from 31% to 64% [6,7] when client-owned cats are examined.

Feline leukemia virus (FeLV) is a gammaretrovirus [8] and feline immunodeficiency virus (FIV) is a lentivirus [9]. Several studies reported that the prevalence of both retroviruses varies among regions [10,11,12,13,14]. Three large prevalence studies have been conducted in North America [15,11,16]. Reported European prevalence ranged from 3.3% to 11.8% [17,8] for FeLV and from 3.2% to 23.6% for FIV [18,19] when client-owned cats were examined.

Epidemiological studies are needed on the prevalence of FCoV, FeLV, and FIV among cats to define and implement prophylactic measures to control the spread of FeLV, FIV, and FCoV infections among the domestic cat population. Data on the prevalence of FeLV, FIV, and FCoV from different parts of the world are necessary to understand the distribution of these viruses worldwide. Croatia is a Mediterranean country. Mediterranean countries have higher levels of FeLV than the rest of Europe [14]. In a study of 6005 cats in Europe the highest prevalence of FeLV infection was in the Southern European countries (5.5%) and the lowest in the Northern European countries (0.7%) [14]. In the study Buch et al., 2017 [13], the highest prevalence of FIV infection was in Southern Europe (12%) and the lowest in Northern Europe (7%). Data on the prevalence of FeLV and FIV in Croatia are limited. In Croatia, FeLV and FIV epidemiological studies have been conducted using serological techniques [20,21]. Therefore, epidemiological research on the prevalence of FeLV, FIV, and FCoV among the population of domestic cats in different areas of Croatia is necessary to develop highly effective prophylactic protocols for owned, stray and feral cats.

The aim of this epidemiological study was to determine the prevalences for anti-FCoV antibody, FeLV antigen, FeLV proviral DNA, and anti-FIV antibody among client-owned cats from the cities of Zagreb and Varaždin in Croatia.

## Materials and methods

Client-owned cats from the cities of Zagreb and Varaždin were studied, but for the purpose of this study, these cats were treated as a single population. Aseptically, 1 mL of whole venous blood from each of 106 individual client-owned cats was sampled into EDTA-coated tubes. Veterinarians took blood samples from domestic cats in veterinary clinics in Varaždin and Zagreb in their daily work from regular patients during practice. No blood was taken from cats for the purpose of this study. The results of blood tests from domestic cats whose blood samples were taken in the diagnosis process during regular veterinary practice were used for this study. Considering that Article 3 of the Animal Protection Act of the Republic of Croatia (Official Gazette 135/06) stipulates that an experiment is one or more procedures performed on an animal for experimental purposes, which may cause pain, suffering or injury to animals, it is clear that the use of blood test results taken for the purpose of determining the patient's health during regular veterinary practice is not an experiment within the meaning of this Act and therefore no ethical approval was required. Samples were stored at 4°C before transporting on dry ice to the Faculty of Veterinary Medicine, Vienna, Austria. Diagnostic tests were conducted at the Faculty of Veterinary Medicine, Vienna, Austria.

### Diagnostic tests

#### Serology testing

##### Detection of FeLV antigen and FIV antibodies cat sera

Serum samples were tested with ELISA (ViraCHEK®/FeLV Synbiotics Corporation, San Diego, CA, USA) for FeLV p27 antigen and with immunomigration test (Witness FIV, Synbiotics Corporation) for anti-FIV antibody. The reported sensitivity and specificity for ViraCHEK®/FeLV test were 94.9% and 98.4%, and for WITNESS FIV test 94.5% and 99.4% [22]. Interpretation of serological test results was performed according to the manufacturer's instructions.

##### ViraCHEK®/FeLV test

###### Test procedure

One well was used for the positive control, one well for the negative control and one well for each sample. One drop or 0.05 ml of Positive Control was added to the first well for positive control and one drop or 0.05 ml of Negative Control to the second well for negative control. 0.05 ml of sample was pipetted into the next well; then one drop or 0.05 ml of Reagent 1 HRP Monoclonal Antibody Conjugate was added to each well. The well holder was tapped (without splashing) for 15 seconds to mix the solutions. After 5 minutes the wells were washed 5 times with deionized/distilled water. After each washing, the holder and blot were firmly inverted onto a paper towel to remove the last drops. Thereafter, two drops or 0.10 ml of Reagent 2, the Chromogenic Substrate Buffer were added to each well. The well holder was then tapped (without splashing) for 15 seconds to mix the solutions. Interpretation of the test results was performed after 5 minutes. The development of a blue color was considered positive for FeLV p27 antigen. A positive test indicates FeLV infection, transient or persistent.

##### WITNESS FIV test

###### Test procedure

50 µl of serum was pipetted vertically into the sample well (1). Three drops of buffer were then added to the sample well (1). The test device remained flat during the migration of the reagent complex sample through the reading window. Interpretation of the test results was performed after 10 minutes. The presence of pink bands in the reading windows (2) and (3) indicated that the sample was positive for FIV antibodies. The presence of anti-gp40 antibodies indicates that the cat has been exposed to the virus.

##### FCoV antibody test

##### Immunofluorescence for antibodies against group 1 coronaviruses

Anti-FCoV antibody and FCoV antibody titers were determined by a modified, indirect immunofluorescence assay [23]. CrFK monolayers grown in 96 well plates were inoculated with Feline Coronavirus 79-1146 (type 2, ATCC VR 990) until carbapenemase-producing enterobacteriaceae was visible (on day 1 or 2) and fixed with

alcohol. For each serum investigated, three dilutions were prepared using phosphate-buffered saline (PBS): 1:10, 1:100, and 1:400. The wells were incubated with 50 µl of the serum dilution for 1 hour at 37°C and washed three times with PBS. 50 µl of a dilution of 1:40 of commercially available fluorescein isothiocyanate (FITC) conjugated goat anti-cat antibodies (Jackson ImmunoResearch) was added to each well. After incubation at 37°C for 1 hour, the wells were washed with PBS and 50 µl of a glycerine-phosphate buffer was added. The plates were evaluated using an inverse UV-microscope. For each sample, the high test dilution with a clear cytoplasmatic fluorescence corresponded to the Coronavirus 1 specific antibody titer. IFA FCoV antibody titeres ranged from 1:10 to 1:400. For FCoV IFA titer <1:10 was indicated as serologically negative, while FCoV IFA titer 1:10 or more as seropositive. An FCoV IFA titer 1:10 was considered low grade positive. A FCoV IFA titer 1:100 was considered medium grade positive, while an FCoV IFA titer ≥1:400 was considered high grade positive.

#### *Real-time PCR for FeLV*

##### Preparation of EDTA blood samples

EDTA blood samples were centrifuged at 3400 x g at 4°C for 15 min. The buffy coat was incubated in erythrocyte lysis buffer (buffer EL, Qiagen, Austria) on ice for 10 min and centrifuged at 470 x g at 4°C for 10 min. The supernatant was discarded and the wash step repeated one or two times until the pellet was visibly white. Finally, the pellet was incubated in 180 µl buffer ATL (Qiagen, Austria) and 20 µl Proteinase K at 56°C until complete lysis.

##### Extraction of viral nucleic acids

140 µl of the leukocyte lysate served as a template for nucleic acid extraction using a commercially available kit (QIAamp® Viral RNA Kit, Qiagen, Austria) as instructed by the manufacturer. Negative controls consisting of the components of the kit were run together with the samples through all sample preparation and extraction procedures. Extracts (60 µl) were stored at -20°C until PCR analysis.

##### Real-time PCR assay for FeLV proviral DNA

The real-time PCR method was conducted as described by Tandon et al. (2005) [24]. DNA was amplified in an Applied Biosystems 7300 Real-Time PCR System, Foster City, CA, USA. PCR reactions were prepared with 12.5 µl qPCR™ Mastermix (Eurogentec, Seraing, Belgium), a final concentration of 480 nM of primers (Microsynth, Balgach, Switzerland), 160 nM of fluorogenic probe (Eurogentec) and 5 µl of the extracted nucleic acid samples in a 25 µl total reaction volume. The RT-PCR mixture was prepared using the components of a commercially available kit (SuperScript™ III Platinum® One-Step qRT-PCR System, Invitrogen) in a reaction volume of 25 µl (2.5 µl template and 22.5 µl PCR mixture). Negative controls (for extraction) and no template controls (for PCR) were run with every assay. Extracts of cell culture supernatants of FeLV (strain FL-237, ATCC VR-721) infected Crandell feline kidney cells served as positive controls. PCR reactions were performed using forward primer FeLV\_U3\_exo\_f: 5'-AAC AGC AGA AGT TTC AAG GCC-3', reverse primer FeLV\_U3\_exo\_r: 5'-TTA TAG CAG AAA GCG CGC G-3' and FeLV\_U3\_probe 5'- CCA GCA GTC TCC AGG CTC CCC A- 3' as described by Tandon et al. (2005) [24].

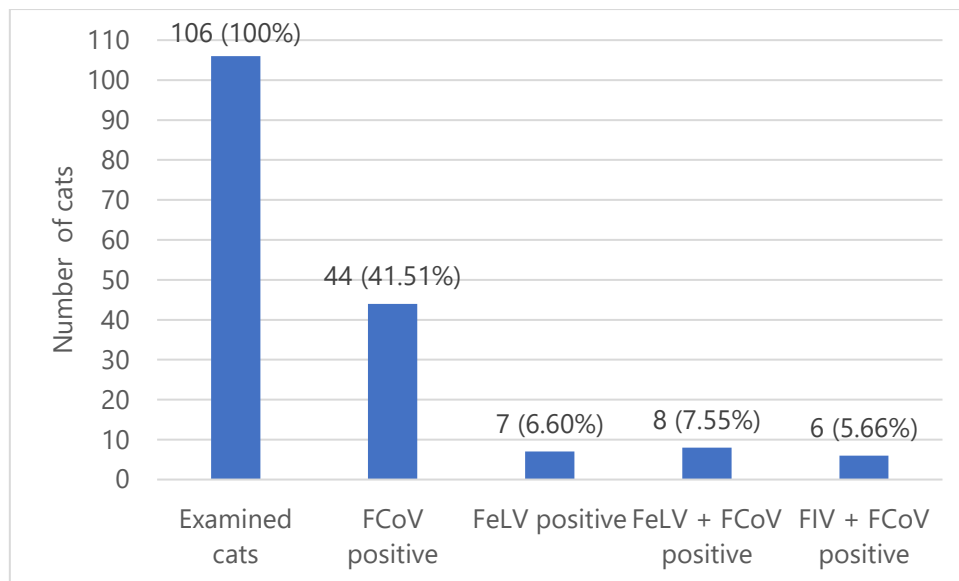
#### **Statistical analyses**

Statistical analyses were carried out in, Excel, (Microsoft Office 2019 Professional Plus)

#### **Results**

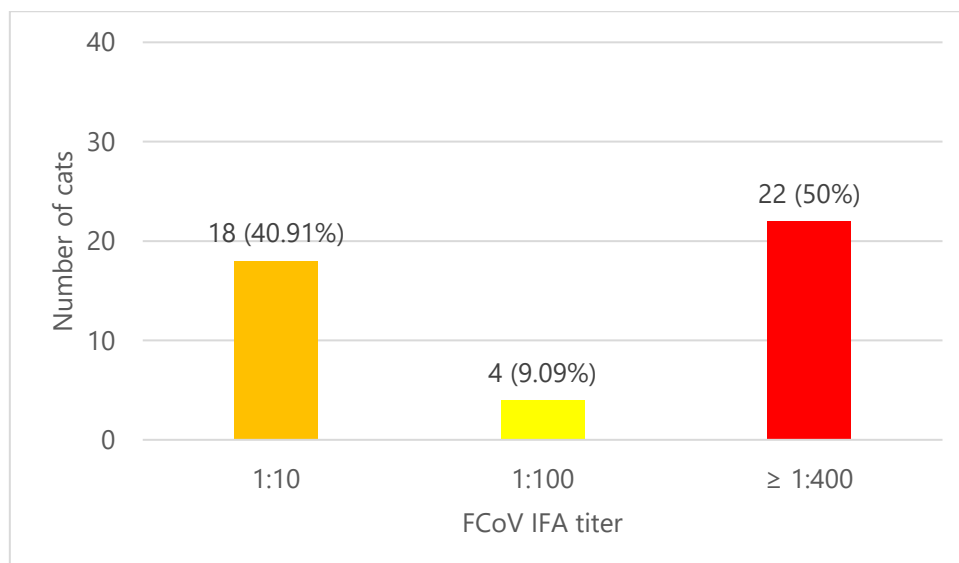
##### **Prevalence**

Anti-FCoV antibodies were detected in 44 (41.51%) of the 106 client-owned cats included in the study (Fig. 1).



**Figure 1: Prevalences of infections caused by FCoV, FeLV, and coinfections caused by FeLV, FCoV, and FIV, FCoV in 106 client-owned cats from the area of Zagreb and Varaždin, Croatia.**

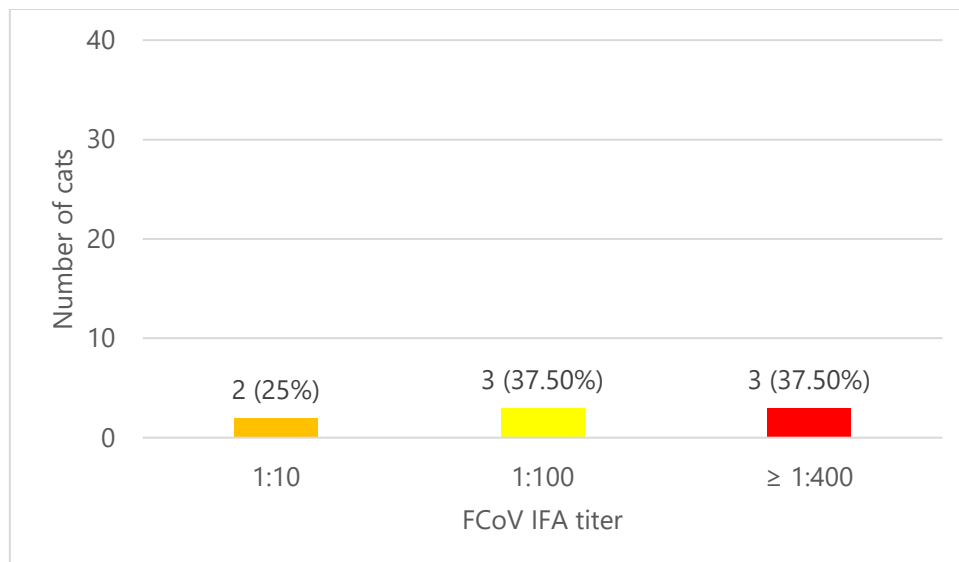
Among those 44 cats, 18 (40.91%) had antibody titers of 1:10, 4 (9.09%) had antibody titers of 1:100, and 22 (50%) had titer of  $\geq 1:400$  (Fig. 2).



**Figure 2: Distribution of FCoV IFA titers among cats (FCoV positive).**

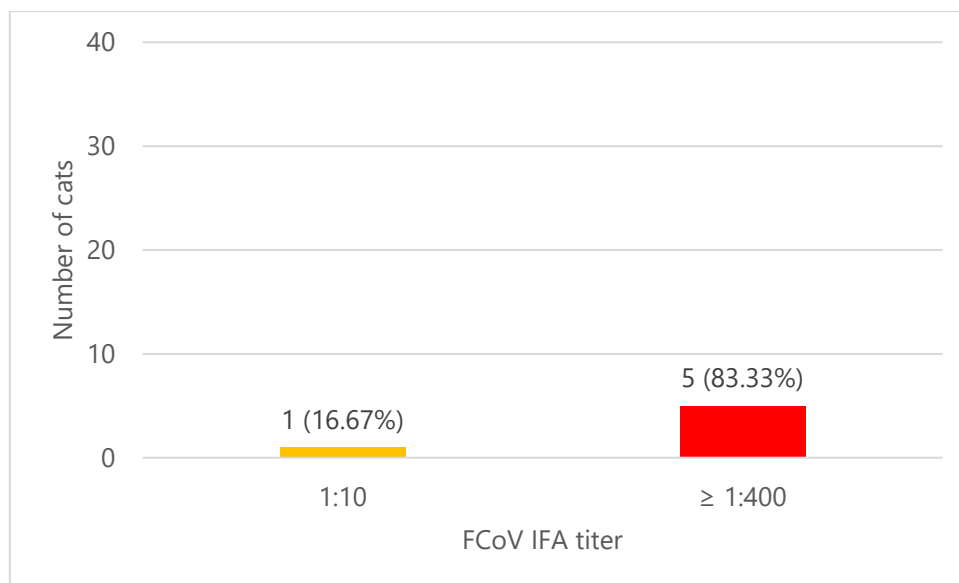
FeLV p27 antigen was detected in 2 of the 106 cats, while FeLV proviral DNA was detected in 4. FeLV p27 antigen and FeLV proviral DNA were detected in 1 of the 106 cats. Of 106 cats tested, 7 (6.60%) cats were FeLV-positive (Fig. 1).

FeLV p27 antigen and anti-FCoV antibodies were detected in 2 of the 106 cats. FeLV p27 antigen, FeLV proviral DNA, and anti-FCoV antibodies were detected in 6. Of 106 cats tested, 8 FeLV-positive cats were positive for anti-FCoV antibodies, yielding a FeLV/FCoV coinfection rate of 8/106 (7.55%); 2 (25.00%) of the 8 cats had antibody titers of 1:10, 3 (37.50%) had antibody titers of 1:100 and 3 (37.50%) had titers of  $\geq 1:400$  (Figs. 1, and 3).



**Figure 3: Distribution of FCoV IFA titers among cats (FeLV+FCoV positive).**

Anti-FIV antibodies and anti-FCoV antibodies were detected in 6 of the 106 cats, yielding an FIV/FCoV coinfection rate of 6/106 (5.66%); 1 (16.67%) of the 6 cats had antibody titer of 1:10, and 5 (83.33%) had titer of  $\geq 1:400$  (Figs. 1, and 4). None of the 6 cats had an antibody titer of 1:100 (Fig. 4).

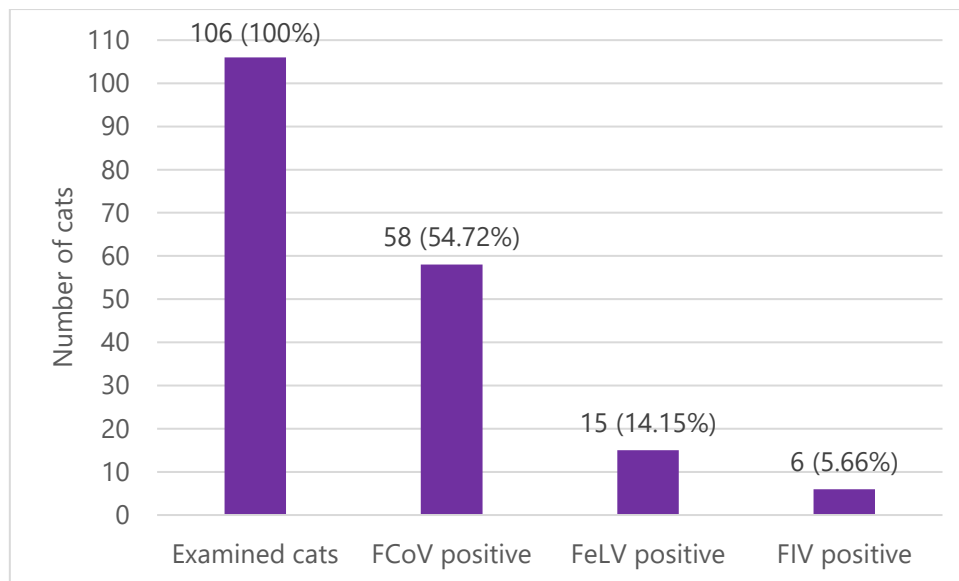


**Figure 4: Distribution of FCoV IFA titers among cats (FIV+FCoV positive).**

54.72% (58/106) of the antibody-positive cats showed varying levels of IFA FCoV-Ab titer of 1:10, 1:100, and  $\geq 1:400$ . The most frequently observed IFA FCoV-Ab titer ( $\geq 1:400$ ) was observed in 50% (22/44) of the FCoV seropositive cats (Fig. 2), in 83.33% (5/6) of the cats coinfecting with FIV and FCoV (Fig. 4), and in 37.50% (3/8) of the cats coinfecting with FeLV and FCoV (Fig. 3).

No cats were coinfecting with FIV and FeLV.

A total of 79 (74.53%) cats were positive for at least one of the 3 viruses tested: the global FCoV, FeLV and FIV prevalences were 54.72%, 14.15%, and 5.66%, respectively (Fig. 5).



**Figure 5: Total prevalence of infections caused by FCoV, FeLV, and FIV in 106 client-owned cats with the inclusion of prevalence data of single infections and coinfections.**

### Discussion

Since the first serological study of FCoV was conducted in Davis, California, USA Pedersen, 1976; [25], many prevalence studies have been conducted in different countries, some of which are shown in (Table 1). Reports indicate that the prevalence of FCoV varies among countries (Table 1). This variability in FCoV prevalence is caused by the geographical location [5,6], different populations of cats [6], breed [4], and climate [25,26].

**Table 1: FCoV prevalence among client-owned cats in different countries.**

Country	Status of cats	Prevalence	Reference
USA	Healthy and ill	35%	Rodgers and Baldwin, (1990) [27]
Israel		21.2%	Baneth et al., (1999) [5]
Czech Republic	Healthy and ill	58%	Moestl et al., (2002) [7]
Austria	Healthy and ill	64%	Moestl et al., (2002) [7]
Switzerland	Healthy	50%	Kummrow et al., (2005) [28]
Sweden	Healthy	31%	Holst et al., (2006) [6]
Australia	Healthy and ill	34%	Bell et al., (2006) [25]
Turkey	Healthy	69.7%	Oguzoglu et al., (2010) [1]
Turkey	Healthy and ill	45.5%	Oguzoglu et al., (2013) [29]
Poland	Healthy and ill	38.5%	Rypula et al., (2014) [30]
Turkey	ill	57%	Tekelioglu et al., (2015) [2]
Japan	Healthy	25.8%	Tsukada et al., (2016) [31]
China	Healthy	72.2%	Li et al., (2019) [32]
Brazil		64.2%	Almeida et al., (2019) [3]

The reported prevalence of FCoV in Poland, Turkey and the United States was 38.5%, 45.5%, and 35% [30,29,27] among owned cats. These results are consistent with the results obtained in the current study (41.51%) (Fig. 1). In contrast, the seroprevalence of FCoV in the current study was higher than observed in Sweden [6], Israel [5], Japan [31], and Australia [25], but lower than in Switzerland [28], Turkey [1,2], China [32], and Brazil [3] (Table 1).

FCoV positive cats were clinically asymptomatic, similar to the results of Sharif et al., (2009) [4], and Oguzoglu et al., (2010) [1]. However, Oguzoglu et al., 2013 [29] reported that health status was significantly associated with FCoV infection and that clinically ill cats were more frequently positive for FCoV than healthy ones.

FCoV is shed in feces by healthy cats and mainly transmitted by fecal-oral transmission [6,26,33]. Infection is most common in multi-cat households in which cats share litter trays with an FCoV-infected cat [6,33]. FCoV infection was confirmed in multiple-cat households in Switzerland [28], Sweden [6], Australia [25], and Poland [30]. Outdoor access reduces the risk of FCoV infection because cats with outdoor access have the ability to bury feces outside and thus minimize oral fecal contact and FCoV transmission [25,34]. Common risk factors for acquiring FCoV infection include the age [29,32,3,34], breed [25,6,26], health status [25,29], multiple cats environment [25,4,32], indoor and/or indoor-outdoor status [3], and climate [25,26].

Researchers investigated a possible correlation between FCoV antibody titer and fecal virus shedding. Pedersen et al., (2008) [35] confirmed a correlation between the shedding of FCoV and antibody titer. However, some researchers reported contrasting results [36,37]. According to a study by Felten et al., (2020) [37], determination of antibody titers in serum or plasma and FCoV viral RNA in fecal samples provide more reliable and accurate results for FCoV status than each test alone.

FeLV was first described in 1964 Jarrett et al., (1964) [38]. Since its discovery, many studies have been conducted on the prevalence of FeLV in various countries and varying prevalence rates have been reported depending on geographical location (Table 2). This variability in prevalence rates is caused by geographical location, lifestyle [18,9,39], health status [18], population density of cats [9,39], prophylactic measures [14], and various diagnostic laboratory methods [40].

**Table 2: FeLV prevalence among client-owned cats in different countries.**

Country	Status of cats	Prevalence	Reference
Israel	Healthy	5.8%	Baneth et al., (1999) [5]
Croatia	Healthy and ill Owned and stray	26.4%	Kučer et al., (2000) [20]
Italy	Healthy	8.4%	Bandecchi et al., (2006) [41]
USA and Canada	Healthy and ill	5.1%	Levy et al., (2006) [15]
Germany	Healthy and ill	3.6%	Gleich et al., (2009) [18]
Canada	Healthy and ill	2.6%	Little et al., (2009) [11]
Turkey	Healthy	12.1%	Oguzoglu et al., (2010) [1]
Malaysia	Healthy and ill	13.1%	Bande et al., (2012) [9]
Turkey	Healthy and ill	20.5%	Oguzoglu et al., (2013) [29]
Poland	Healthy and ill	6.4%	Rypula et al., (2014) [30]
Mexico	Healthy	7.5%	Ortega-Pacheco et al., (2014) [42]
Iran	Healthy and ill	12.22%	Torkan et al., (2014) [43]
Austria	Healthy and ill	5.6%	Firth and Möstl (2015) [44]
Australia	Healthy and ill	2%	Westman et al., (2016) [12]

Japan	Healthy	2.5%	Tsukada et al., (2016) [31]
China	Healthy and ill	9.80%	Cong et al., (2016) [45]
Cyprus	Sick	6.9%	Attipa et al., (2017) [46]
USA and Canada	Healthy and ill	3.1%	Burling et al., (2017) [16]
New Zealand	Healthy and ill	2.6%	Luckman and Gates (2017) [47]
Croatia	Sick	18.22%	Perharić et al., (2018) [21]
Serbia	Healthy and ill	1.3%	Sarvani et al., (2018) [19]
Switzerland	Healthy and ill	2%	Hofmann-Lehmann et al., (2018) [48]
Thailand	Healthy	3.1%	Aiyaranoi et al., (2018) [49]
Malaysia		12%	Sivagurunathan et al., (2018) [50]
Brazil	Healthy and ill	5.8%	Rocha et al., (2019) [51]
Croatia	Owned and stray	4.5%	Studer et al., (2019) [14]
Hungary	Healthy and ill	11.8%	Szilasi et al., (2019) [8]
Italy		4.3%	Iatta et al., (2019) [52]
Ireland	Healthy and ill	3.3%	Szilasi et al., (2021) [17]

The prevalence of FeLV infection observed in the current study (Fig. 1) was similar to the results of studies from Italy [52,41], Austria [44], Poland [30], Cyprus [46], Israel [5], Brazil [51], and Mexico [42] (Table 2). In the current study, the FeLV prevalence was higher than observed in Ireland [17], Germany [18], Serbia [19], Switzerland [48], USA and Canada [16], Japan [31], Thailand [49], Australia [12], and New Zealand [47], but lower than in Hungary [8], Turkey [1,29], Malaysia [50,9], Iran [43], and China [45] (Table 2).

FeLV infection is transmitted via the oro-nasal route through mutual grooming and sharing of food and water dishes [20,18,53]. The majority of FIV infections are transmitted by bites [20,18,53]. Risk factors for acquiring FeLV and FIV infection include male gender [18,16], being not neutered [53], outdoor access [18,9,53], fighting [18,11,9,53,16], and adulthood [11,18,53,16]. Fighting and aggressive behavior are more frequently expressed among more aggressive male cats [18,9]. For example, one study in Malaysia Bande et al., 2012 [9] reported that cats with aggressive behavior were 2 times more likely to test positive for FIV (46.0%) or FeLV (20.7%) compared to non-aggressive cats (26.7%; 9.6%). However, risk factors are variable in epidemiological studies. For example, in a study conducted by Gleich et al., 2009 [18], the risk of FeLV infection was significantly higher in male (62%) than in female (38%) cats. Contrastingly, Bandecchi et al., 2006 [41] observed no significant association between gender and seropositivity for FeLV.

Among the FeLV positive cats, only two cats were clinically ill. Except for those cats, 5 of 7 cats (71.43%) were clinically healthy, indicating that these cats were asymptomatic viraemic cats. This finding indicates that it is important to test not only sick cats but also healthy cats to control the spread of FeLV infection among the domestic cat population. However, some other studies reported a significant correlation between infection status and health status. In studies provided by Bande et al., (2012) [9] and Westman et al., (2016) [12] the positivity rates for FeLV infection were significantly higher among sick cats.

The immunosuppression caused by FeLV and FIV [5,9] increases the risk of coinfections, such as FCoV [1], FIP [29], Bartonella henselae (BH), Toxoplasma gondii, Leishmania infantum [54] or Dirofilaria immitis [45]. Approximately up to 12% of FCoV infected cats develop feline infectious peritonitis (FIP) [2]. Coinfections of FeLV/FCoV and FIV/FCoV were confirmed among owned and stray cats worldwide as shown in Tables 3 and 4.



**Table 3: Prevalence of coinfection with FeLV and FCoV among client-owned cats in different countries.**

Country	Status of cats	Prevalence	Reference
Israel	Owned and stray	1%	Baneth et al., (1999) [5]
Turkey	Healthy Owned and stray	5.66%	Oguzoglu et al., (2010) [1]
Italy	Stray	4.8%	Spada et al., (2016) [54]

**Table 4: Prevalence of coinfection with FIV and FCoV among client-owned cats in different countries.**

Country	Status of cats	Prevalence	Reference
Israel	Owned and stray	5%	Baneth et al., (1999) [5]
Turkey	Sick Owned and stray	5%	Tekelioglu et al., (2015) [2]

In this study, the prevalence of coinfection with FeLV and FCoV (7.55%) (Fig. 1) was higher than reports from Israel [5], Turkey [1], and Italy [54] (Table 3).

Among cats with FeLV and FCoV, six of 8 cats were clinically ill (6/8; 75.00%). This result is inconsistent with the study in Turkey that reported that all cats with FeLV and FCoV were clinically healthy [1].

In the current study, all FIV-infected cats were seropositive for anti-FCoV antibody (5.66%) (Fig. 1) and seroprevalence of coinfection with FCoV and FIV was very similar to results of previous studies [5,2] (Table 4). All cats coinfecting with FCoV and FIV were clinically ill in the current study, which is consistent with the study by Tekelioglu et al., (2015) [2].

Based on the data obtained in the current research, the rate of FeLV infection was 14.15% with the inclusion of the FeLV positivity rates in single infection (6.60%) and in coinfection (7.55%) (Figs. 1, and 5). This finding supports observations from a previous Croatian study [21], but not the most recent data from European countries (with the exclusion of prevalence data including stray or shelter cats). FeLV prevalence was higher than observations of studies from Germany [18], Poland [30], Austria [44], Switzerland [48], Italy [52], and Ireland [17].

Additionally, the rate of FCoV infection was 54.72% with the inclusion of the FCoV positivity rates for single infections (41.51%) and in coinfections (13.21%) (Figs. 1, and 5). The seroprevalence of antibodies against FCoV in Croatia is similar to those in central European countries. In a study by Moestl et al., (2002) [7] FCoV seroprevalence was 58% in the Czech Republic and 64% in Austria.

The important results from the current study are that the prevalence of FCoV and FeLV is high, FIV is rarer in the Croatian cat population (Fig. 5). The prevalence of FCoV infection was higher than that of FeLV infection (Fig. 5), similar to results of studies of owned cats in Turkey [1,29], and in Poland [30]. In this study, the prevalence of the FeLV infection was higher than that of the FIV infection (Fig. 5), similar to results of studies of owned cats in Germany [18], in Turkey [29], in Poland [30], in Mexico [42], in Hungary [8], and in Italy [52]. However, some other studies in Israel [5], Australia [12], in New Zealand [47], in Cyprus [46], in Brazil [55], and in Ireland [17] reported contrasting results.

Previous study has reported a high rate of retroviral infections in stray cats from the Zagreb metropolitan area in Croatia [21]. The high-density cat population and the large number of stray cats in urban areas increase the chance of contact with other possible FeLV and/or FIV infected stray and free roaming client-owned cats and increase more fighting and aggressive behavior among adult male cats. Therefore, outdoor owned cats are at higher risk of retroviral infections due to more frequent exposure to these viruses. Factors contributing to the high rate of FeLV infection in owned cats and to the FIV infection risk of client-owned cats in the current study may be the high density of cat population and the large number of stray cats living in the urban areas.

Testing, identification of infected cats and vaccination are essential for retroviral prevention of transmission [56]. Recommendations for the use of available FeLV vaccines have been published [56]. Vaccination and testing have caused a decrease in the prevalence of FeLV infection [14]. Recommendations for the use of FIV vaccines have also been published, but variability in vaccine efficacy has been reported [56]. Data regarding the vaccination status and testing for feline retroviral and coronavirus infections collected along with the sampling of cats, indicate that preventive measures should include testing for FeLV, FIV, and FCoV, segregation of FeLV and FIV infected cats, and vaccination against FeLV in Croatian cat populations, as among the cats included in this study, none had received vaccination against the feline leukemia virus, 0.94% were tested for FeLV antigen, and 1.89% were tested for anti-FIV antibody.

## Conclusions

This study confirmed the presence of FCoV, FeLV, and FIV infections and coinfections with FeLV/FCoV and FIV/FCoV in the cities of Zagreb and Varaždin. The seroepidemiological findings from this study demonstrate that both feline retroviruses and feline coronavirus are important feline pathogens in the owned cat population in the cities of Zagreb and Varaždin. The results further indicate the importance of preventive testing of cat samples for anti-FCoV antibody, FeLV antigen, FeLV proviral DNA, and anti-FIV antibody to detect the presence of FCoV, FeLV, and FIV infections in the cat population. Preventive measures should include testing, identification of infected cats, segregation and vaccination against FeLV to control these significant infections in owned cats in urban areas in northwestern Croatia. The findings obtained in this study should be helpful for the development of further prophylactic protocols for owned cats in urban areas in Croatia and neighboring countries. However, the results of the current study should be interpreted with caution. The limitations of this research are: a small number of cats and different cat populations (owned, stray, feral cats) from different Croatian areas were not included in this study. Therefore, further more detailed studies with a larger number of owned, stray and feral cats from different urban areas in Croatia are needed to determine the prevalence of FCoV, FeLV, and FIV infections and risk factors for seropositivity among cats in Croatia. The results of this study highlight that data on the prevalence of FCoV, FeLV, and FIV in a larger number of cat samples from different Croatian areas are essential for the development of a prophylactic protocol and for the implementation of effective prevention measures for client-owned, stray and feral cats.

## Data Availability Statement

The data (materials and methods, results, (Figs 1-5) used in this research are based on the author's doctoral dissertation Raukar, J. (2016). Сероепидемиолошко истраживање инфекција коронавирусом, вирусом леукемије и вирусом имунодефицијенције мачака у Републици Хрватској, *Универзитет у Новом Саду* which was defended at the University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia. Doctoral dissertation is openly available in the НаРДУС Национални Репозиторијум Дисертација у Србији (NaRDuS National Repository of Dissertations in Serbia) at <https://nardus.mpn.gov.rs/handle/123456789/8747>

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## Conflicts of interest

The author declares that there is no conflict of interest.

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