Abstract
Feline coronavirus (FCoV) is ubiquitous in the domestic cat (Felis catus) population. The aim of this study was to determine the prevalence and potential predisposing factors of FCoV in cats of an animal shelter in Latvia and to compare the prevalence between cats in the quarantine area and resident cats in the adoption area. Oropharyngeal and faecal swabs and blood samples were collected from 40 domestic shorthair cats from an animal shelter in Jelgava, Latvia. Swabs were analyzed for FCoV RNA by reverse transcriptase-polymerase chain reaction (RT-PCR). Blood serum samples were tested for FCoV specific antibodies by indirect enzyme-linked immunosorbent assay (ELISA). FCoV RT-PCR positivity in oropharyngeal and rectal swabs was 7.5% (3/40) and 72.5% (29/40), respectively. Additionally, FCoV seroprevalence was 67.5% (27/40). The proportion of cats shedding FCoV within the adoption (72.7%) and quarantine (72.2%) areas was similar (p = 0.55). The prevalence of FCoV faecal shedding in young cats was significantly higher (p < 0.05) than in adult cats. Sex had no significant effect on FCoV RT-PCR positivity. Further studies on larger cat population including different population types are needed to determine the overall prevalence and epidemiological patterns of FCoV infection in Latvia.

Key words: coronavirus (CoV), feline infectious peritonitis (FIP), feline coronavirus (FCoV), cats, animal shelter, RT-PCR.
Materials and Methods

Study population

Samples from 40 domestic shorthair cats (22 male and 18 female) were collected from an animal shelter in Jelgava, Latvia, between September 2020 and January 2021. Examined cats were housed in a quarantine zone for incoming cats and in an area designated for resident cats. Age was provided by the owners who surrendered their cats or estimated by shelter staff in stray cats. The youngest cat tested was 2 month old, but the oldest was 12 years old; they were categorized as juvenile (<1 year) and adults (≥1 year). The clinical condition of all cats enrolled in this study was not an excluding factor. The study was approved by the Committee for the Protection of Animals Used for Scientific Purposes of the Food and Veterinary Service of the Republic of Latvia (certificate of approval No 119).

Sample collection

From each cat two polyester-tipped swabs (oropharyngeal and rectal) and one blood sample were collected. No anesthetic when collecting the samples was used, only proper handling, and a minimum of physical restraint. For swab collection, transport, and maintenance UTM™ paired with COPAN FLOQ Swabs® were used. Commercial UTM™ conical tubes were filled with 3 mL UTM™ medium. Half of the viral transport medium was poured into sterile 1.5 mL Eppendorf Tubes® before taking the swabs. For the collection of oropharyngeal specimen, the minitip size swab was inserted into the caudal oropharynx and tonsillar areas. The sample was collected by rubbing the polyester-tipped shaft against the caudal oropharynx while trying to avoid contact with the tongue, teeth, and gums. Then the swab was inserted into the UTM™ tube until the breakpoint was level with the tube opening and the swab shaft was broken off at the breaking point. The second regular size swab was inserted 1.0–1.5 cm into the rectum. The sample was collected by gently rolling the swab against the rectal mucosa, then the swab was inserted in the pre-filled Eppendorf Tube® and the shaft was cut leaving a tip of the swab into viral transport medium. For blood collection vacuum tube with clot activator and 23-gauge butterfly catheter were used. The cephalic vein was occluded with a tourniquet, the venipuncture site was clipped and cleaned with 70% isopropyl alcohol pad. Subsequently, the butterfly catheter was inserted intravenously. From each cat about 0.7 to 1.5 mL of peripheral blood was obtained. Then the tourniquet was released, the catheter was withdrawn and pressure to the puncture site was applied.

Serological testing

All blood serum samples were tested for the presence of specific antibodies to FCoV by an indirect enzyme-linked immunosorbent assay (ELISA) using a commercial INGEZIM CORONA FELINO indirect ELISA kit (Ingenasa, Spain). The procedure of the test was carried out according to the manufacturer’s instructions. Briefly, a commercial plate is already coated with the specific FCoV antigen. For the reaction 200 µL of serum dilution 1/200 were added to the plate and incubated. If the samples contained specific antibodies to FCoV, they bound to the antigen. The plate was washed and the specific peroxidase conjugate was added. The second washing of the plate followed and the substrate was added to the wells. The Multiskan FC® spectrophotometer (Life Technologies, Singapore) was used to measure the optical density (OD) of the colorimetric reaction. The cut-off value was determined according to the user manual of the kit. The samples with an OD value higher than the cut-off were considered positive and having specific antibodies to FCoV. The samples with an OD value lower than the cut-off were considered as negative for the presence of specific antibodies to FCoV.

Detection of FCoV by reverse transcriptase-polymerase chain reaction (RT-PCR)

Viral RNA was extracted from 200 µL of sample using the IndiSpinPathogen kit (INDICAL, Leipzig, Germany). The RNA was eluted in 100 µL of elution buffer and stored at −80 °C. CoV screening was performed by a pan-coronavirus one-step RT-PCR followed by sequencing of the amplified product (440 bp) to confirm CoV identification. PCR was performed by adding 5 µL of extracted RNA to 20 µL of the SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA polymerase kit (Invitrogen, Carlsbad, USA) reaction mixture containing 0.5 µM of each primer (RdRP2-F GTGTGGGACTATCTAAGTGTA and RdRP2-R CCATCATCAGATAGAATCATCATA) (Poon et al., 2005). RT-PCR was carried out at 50 °C for 30 min, followed by the activation of the DNA polymerase at 95 °C for 2 min, and by 40 cycles in three steps: 95 °C for 30 s, 50 °C for 30 s, and 68 °C for 1 min. An additional extension for 10 min at 68 °C was added at the end of the run. The RdRp PCR products were enzymatically purified using Exol and FastAP (Life Technologies, Fermentas, Lithuania) and were subjected to nucleotide sequence analysis.

Statistical methods

Descriptive statistics for the age of the cat, sex, area in the shelter and FCoV status in all sample types was calculated using Student’s t-tests for unpaired samples. P-values of less than 0.05 were considered statistically significant. Additionally, the Pearson correlation coefficient was calculated for RT-PCR and ELISA results.

Results and Discussion

A total of 40 cats from a single shelter were included in the study, of those 22 (55%) resided in the
The overall prevalence of previous or ongoing FCoV infection was 82.5% (33/40). Out of the 40 cats, 29 cats (73%) were shedding FCoV, but 27 cats (68%) had anti-FCoV antibodies. There was no significant difference ($p = 0.55$) between the proportion of cats shedding FCoV within the adoption (72.7%) and quarantine (72.2%) areas. These results demonstrate that compartmentalization of the particular shelter into individual sections did not reduce transmission of FCoV and different prevention strategies for FCoV infection should be applied. Shedding cats are responsible for the persistent presence of FCoV in the domestic cat population (Felten et al., 2020) therefore, in order to avoid continual reinfection, shedders must be isolated.

In previous studies estimated FCoV seroprevalence in multicat environment was highly variable, ranging from 25.6% in the United Kingdom (Cave et al., 2004) to 82% in Italy (Pratelli, 2008) depending on various factors such as population density, husbandry practices, time spent in the shelter before sampling, age, breed, and health status (Cave et al., 2004). Faecal shedding, as determined by RT-PCR from rectal swabs, in previous studies with mixed-breed cats in multicat environment was similar to our results. The overall prevalence of FCoV infection in the cat population in Malaysia and Germany was 70% (Sharif et al., 2009) and 76.5% (Klein-Richers et al., 2020), respectively.

The whole study population consisted of 22 (55%) neutered males and 18 (45%) neutered females. We found no significant correlation between FCoV-positivity and the sex of the cats ($p = 0.55$). FCoV prevalence in male cats (72.7%) was almost the same as in female cats (72.2%). Among the 29 FCoV-positive cats, 13 (45%) were less than 1 year old and 16 (55%) were older than 1 year. Prevalence of FCoV fecal shedding in young cats (92.9%) was significantly higher ($p < 0.05$) than in adult cats (61.5%) (Table 1). We observed that young age was the only significant factor associated with FCoV shedding (RT-PCR positivity in rectal swabs). Our findings support previous studies that found a significant correlation between FCoV-positivity and the young age but no significant association between FCoV-positivity and sex of the cats (Klein-Richers et al., 2020; Pedersen, Allen, & Lyons, 2008).

FCoV RNA was detected in 3/40 (7.5%) oropharyngeal swabs and in 29/40 (72.5%) rectal swabs but FCoV specific antibodies in 27/40 (67.5%) blood serum samples. The proportion of positive rectal swabs potentially could be even higher because only a single rectal swab from each cat was analyzed and according to previous studies, 70–80% of infected cats are intermittent shedders (Klein-Richers et al., 2020). Overall, 85% (23/27) of FCoV specific antibody-positive cats shed FCoV in their faeces. There was a moderate positive correlation between the presence of FCoV specific antibodies and FCoV shedding in feces ($r = 0.41$, $p < 0.01$). This observation is in accordance with previous reports which showed that cats with antibodies were more likely to be the virus shedders than non-shedders (Felten et al., 2020; Pedersen, Allen, & Lyons, 2008).

However, the prevalence of FCoV in shelter cats cannot be extrapolated to the overall cat population of Latvia. Further studies on larger cat population including different population types are needed to determine the overall prevalence and epidemiological patterns of FCoV in Latvia.

### Conclusions

1. In this study, the prevalence of previous or ongoing FCoV infection in cats of an animal shelter in Latvia was 82.5%; additionally, young age was a predisposing factor for FCoV infection.

2. The difference between cats shedding FCoV within the adoption (72.7%) and quarantine (72.2%) areas was not significant ($p = 0.55$), thereby new prevention strategies should be applied to reduce FCoV infection and control FIP outbreaks since compartmentalization did not decrease the spread of FCoV.

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**Table 1**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Category</th>
<th>RT-PCR rectal swabs (FCoV RNA) (No. Positive/Total samples)</th>
<th>RT-PCR oropharyngeal swabs (FCoV RNA) (No. Positive/Total samples)</th>
<th>Indirect ELISA serum samples (FCoV specific antibodies) (No. Positive/Total samples)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>12/22</td>
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<td></td>
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<td>13/18</td>
<td>2/18</td>
<td>15/18</td>
</tr>
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<td></td>
<td>≥1 year</td>
<td>16/26</td>
<td>3/26</td>
<td>18/26</td>
</tr>
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</table>
3. A follow-up study should be performed to investigate the development of FIP in conjunction with RNA sequencing of FCoVs to identify specific mutations in the FCoV genome.

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References