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FELINE CORONAVIRUS: AN EPIDEMIOLOGICAL RETROSPECTIVE STUDY IN BUCHAREST CITY, ROMANIA

FELINE CORONAVIRUS: STUDIU EPIDEMIOLOGIC RETROSPECTIV ÎN BUCUREȘTI, ROMANIA

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ABSTRACT | REZUMAT

Coronaviruses, particularly Feline Infectious Peritonitis (FIP) and Feline Enteric Coronavirus (FECV), remain significant issues in veterinary medicine, affecting the health of domestic cats worldwide.FIP, caused by mutations of FECV, is often fatal, with rapid progression in immunocompromised or young cats. FCoV has two main serotypes, FCoV1 and FCoV2, with FCoV2 originating from a combination of FCoV1 and Canine Coronavirus. Feline Coronavirus (FCoV) spreads primarily through the faecal-oral route and is highly endemic in multi-cat environments. Recent studies show an increase in FIP cases, particularly among indoor cats, due to higher exposure to faecal-borne coronavirus. The virus's genetic structure includes three membrane proteins and several accessory proteins linked to FIP development. The study aimed to analyse FIP incidence in southeastern Romania over four years, using real-time PCR for diagnosis. In this study, a total of 977 samples tested for Coronavirus using the real-time PCR method were included, of which 258 were positive for FCoV. Males and European-breed cats were more commonly affected. Despite low viral loads in many samples, the high spread of FCoV indicates significant regional prevalence. Further research is needed to understand breed- and sex-related predispositions fully.

Keywords: Feline Coronavirus, Real-Time qPCR, Diagnostic Feline Coronavirus, FIP prevalence

Coronavirozele, în special Peritonita Infecțioasă Felină (FIP) și Coronavirusul Enteric Felin (FECV), rămân probleme semnificative în medicina veterinară, afectând sănătatea pisicilor domestice la nivel global. FIP, cauzată de mutații ale FECV, este fatală în lipsa unui tratament specific, cu o progresie rapidă în cazul pisicilor imunocompromise sau tinere. FCoV are două serotipuri principale, FCoV1 și FCoV2, FCoV2 provenind dintr-o combinație între FCoV1 și Coronavirusul Canin. FCoV se răspândește în principal prin calea fecal-orală și este extrem de endemic în medii cu mai multe pisici. Studiile recente arată o creștere a cazurilor de FIP, în special în rândul pisicilor de interior, datorită unei expuneri mai mari la coronavirusul transmis prin fecale. Compoziția genetică a virusului include trei proteine de membrană și mai multe proteine accesorii legate de dezvoltarea FIP. Studiul a avut ca scop analiza incidenței FIP în sud-estul României pe o perioadă de patru ani, utilizând PCR în timp real pentru diagnostic. În acest studiu un total de 977 de probe testate pentru Coronavirus, prin metoda real time PCR au fot luate în studiu, dintre care 258 au fost pozitive pentru FCoV. Pisicile de sex masculin și cele de rasă europeană au fost mai frecvent afectate. Deși încărcătura virală a fost scăzută în multe dintre probe, răspândirea ridicată a FCoV indică o prevalență semnificativă la nivel regional. Este necesară o cercetare suplimentară pentru a înțelege pe deplin predispozițiile legate de rasă și sex.

Cuvinte cheie: Coronavirus Felin, Diagnostic Coronavirus Felin, Prevalența PIF

Although discovered many years ago, coronaviruses continue to raise numerous questions both in human scientific communities and in veterinary medicine. Feline coronaviruses, particularly Feline Infectious Peritonitis (FIP) and Feline Enteric Coronavirus (FECV) infections, represent a major issue in veterinary medicine, significantly impacting the health of domestic cats and the global veterinary ecosystem. These diseases are caused by viruses from the *Coronaviridae* family and are characterised by complex pathogenicity, being associated with a wide range of clinical manifestations, from asymptomatic forms to severe conditions, often with an unfavourable prognosis, especially in the case of FIP.

The first reports regarding feline coronavirus were made by Holzworth and date back to 1963, describing a condition in cats with immune-mediated vasculitis and a pyogranulomatous inflammatory response. It was not until 1978 that a virus was identified as the etiological agent. In 1979, it was characterised as a coronavirus and was considered the Feline Infectious Peritonitis Virus, as described by Hartmann in his 2005 article. Coronaviruses, which have become the subject of numerous studies in recent years, form a vast taxonomic tree within the order *Nidovirales* (from the Latin word *nidus* meaning nest) (5).

The 2022 viral taxonomy (EC 54, Online meeting, July 2022, Email ratification March 2023, MSL #38)

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organises the order *Nidovirales* into eight viral suborders: *Abnidovirineae, Arnidovirineae, Cornidovirineae, Mesnidovirineae, Monidovirineae, Nanidovirineae, Ronidovirineae*, and *Tornidovirineae*. The *Coronaviridae* family belongs to the *Cornidovirineae* suborder and is divided into three subfamilies: *Letovirinae*, *Orthocoronavirinae* (referred to as CoV), and *Pitovirinae*. (https://ictv.global/taxonomy).

Alphacoronaviruses include TGEV (Transmissible Gastroenteritis Virus - affecting pigs), PRCV (Porcine Respiratory Coronavirus), CCoV (Canine Coronavirus), which causes a gastroenteric viral infection in dogs, and FCoV (Feline Coronavirus, including two serotypes) (5). FCoV is widespread globally and generally causes mild gastroenteritis symptoms, although about 5% of cats may develop a form of systemic infectious peritonitis (17).

These viruses are closely related. An example of this is the Canine Coronavirus II variant, which originates from CCoV II and TGE. FCoV has two serotypes – FCoV1 and FCoV2, with variant 2 originating from the combination of FCoV1 and CCoV (15). FCoV I and FCoV II are differentiated based on antigenicity using seroneutralisation reactions.

Decaro et al. (2021) divides feline coronavirus serotypes into two biotypes: FECV (Feline Enteric Coronavirus) and FIPV (Feline Infectious Peritonitis Virus) (9). These are genetically related but antigenically distinct (20). Serotype I is most commonly found in Europe, but it has also been reported in Japan, while serotype II is more frequently isolated in Asia. FCoV has 2 serotypes – FCoV1 and FCoV2 and the second variant is actually a combination between FCoV1 and CCoV (15). The virus primarily spreads through the faecal-oral route, and many cats in multi-cat environments, such as shelters and catteries, become infected without showing clinical symptoms (18).

While the majority of FIP cases are observed in young or immunocompromised cats, FECV infection can also affect adult cats, particularly in shelters or environments with a high animal density. In the case of FIP, the disease progresses rapidly and often leads to the animal's death, despite treatment efforts. In breeding catteries, a study conducted by Felten et al. (2023) found that 19% of cats were classified as intermittent shedders, with fluctuating detection of FCoV RNA in four faecal samples collected at intervals ranging from 5 to 28 days. In another study involving pet cats, 31% were either intermittent shedders or had recovered and been re-infected (2). This particular study was notable for its long follow-up period, lasting up to five years.

The virus is commonly found in environments where many cats are confined in close quarters, such as catteries, shelters, and pet stores. It is nearly impossible to find a multi-cat household without endemic FCoV. At least 50% of cats in the United States and Europe carry antibodies against coronaviruses. In Switzerland, 80% of breeding cats and 50% of free-roaming cats tested positive for antibodies. In the United Kingdom, 82% of show cats, 53% of cats in breeding facilities, and 15% of cats in single-cat households had antibodies (13, 21).

In recent years, FIP has surpassed infection with feline leukemia virus (FeLV) as the leading cause of death due to infectious diseases in pet cats. Changes in the management of domestic cats may have contributed to an increase in the prevalence of FIP. The increase in indoor cats has significantly heightened exposure to coronavirus from faeces, a situation that would be less likely if felines were allowed to bury their faeces in the ground and did not share litter boxes (3).

Since the 1990s, substantial research has been conducted on the genetic link between FECV and FIPV. While FECV typically causes mild gastrointestinal illness, FIPV results from a mutation of FECV in infected cats, which can lead to a more virulent and systemic form of the disease. FIP is characterised by significant inflammation in the body cavities, particularly in the peritoneum (abdomen), and is almost always fatal if left untreated (18).

Coronaviruses encode three membrane proteins in their genome: spike (S), which creates the electron microscopic crown-like appearance; envelope (E); and membrane (M), as well as a nucleoprotein (N) (8). These four proteins appear in the order S–E–M–N in any known coronavirus (23). The genome also includes 11 Open Reading Frames (ORFs) and 7 non-structural proteins, including two replicases (1a and 1b) and 5 accessory proteins (3a, 3b, 3c, 7a, and 7b) (11). Two-thirds of the genome is represented by ORFs 1a and 1b, which encode the virus's replication machinery. ORFs 2, 4, 5, and 6 encode the S, E, M, and N proteins, respectively. ORFs 3 and 7 encode the accessory proteins 3a, 3b, 3c, 7a, and 7b, whose functions are not yet fully elucidated. According to the studies by Bank-Wolf et al. (2014), mutations occurring in the accessory protein 3c and the S protein are associated with the development of feline infectious peritonitis (1, 4, 6, 12, 13, 19).

The pathogenesis of FECV and PIF cannot be discussed separately. Thus, after a cat is infected with FCoV through ingestion (or, in rare cases, through inhalation), the intestinal epithelium is the primary site of viral replication. The specific receptor for FCoV (at least for FCoV serotype I) is the enzyme aminopeptidase-N, located at the brush border of the intestinal epithelium (14, 22). Once the virus acquires a tropism for monocyte-macrophage, FCoV can spread systemically, although not all strains with systemic spread are pathogenic. Sometimes, the host's cellular immune response is strong enough to neutralise the virus (7, 18). At other times, the virus reaches the serosal surfaces, lymph nodes, or even the meninges or eyes, causing systemic and organ-specific reactions (18).

The main aim of this study is to conduct a retrospective assessment of the incidence of feline infectious peritonitis in the southeastern region of Romania over a period of 4 years. The definitive diagnosis was made based on real-time PCR, using various biological samples, depending on the clinical stage of the patient.

MATERIALS AND METHODS

Animals and biological samples

Between January 2021 and November 2024, samples from cats suspected of being infected with Feline Coronavirus were tested. The samples from January 2021 until August 2022, 352, were tested in the Molecular Biology Laboratory of the Veterinary Hospital „Prof. univ. Dr Alin Birtoiu" Bucharest. After this date and until December 2024, 572 samples taken in the study were analysed at Histovet Laboratory, also in Bucharest. The protocols used for diagnostics were similar for all the steps of the PCR reaction in both laboratories. A total of 944 samples from cats were tested by the real-time PCR method. in both A & B laboratories. The samples were represented by various matrices, such as puncture fluids from the abdominal or thoracic cavity, whole blood with EDTA, or faeces.

Real-Time PCR

The real-time PCR Method is a very precise and sensitive method of diagnosis. The principle consists of amplification of a certain sequence of nucleotides, specific for each pathogen, using specific primers.

The RNA extraction

It was performed manually by using the QIAamp cador Qiagen kit. This is a non-specific step in the sense that, in the end, all categories of RNA in the biological sample are isolated.

The main steps, as described in the genetic material extraction protocol, following the manufacturer's instructions, were as follows:

1. Sample preparation (in this case, swabs) by adding a lysis buffer solution (Buffer ATL), which helps break the cell membranes and release the genetic material.

2. Addition of Proteinase K, which facilitates the digestion of proteins.

3. The mixture is incubated at the specified temperature (typically 56°C) to allow complete lysis of the samples and the release of RNA or DNA.

4. Isopropyl alcohol (usually 100%) is added to assist in precipitating DNA/RNA. The mixture is gently vortexed to ensure thorough mixing.

5. The mixed sample is passed through the QIAamp Mini column from the kit, which contains a material that binds to DNA or RNA.

6. By centrifugation, the DNA/RNA binds to the column material, and contaminants are washed away through additional centrifugation steps.

7. Specific washing buffers, such as Buffer AW1 and AW2, are added to remove any remaining impurities on the column. After each washing buffer application, centrifugation is performed to ensure complete removal of contaminants.

8. The purified DNA/RNA is eluted from the column using an elution buffer, typically Buffer AE (or another specified elution buffer). Elution was performed with 50 µL of buffer solution, centrifuged at approximately 8000 rpm, and the eluted product was the purified DNA/RNA.

The most commonly used technologies for RNA extraction are based on the ability to selectively bind silica membranes / adsorption of nucleic acids on the surface of paramagnetic particles. For these samples, both methods were used alternatively. The amount of sample necessary to perform the test is at least 200 µL of EDTA blood or ascitic liquid or other biological liquid. For faeces, rectal swabs were used. For all the extracted samples, real-time PCR for Coronavirus was performed, using the same specific primers, regardless of the biological specimen. All the obtained Ct values were recorded, along with the epidemiological data (breed, age, and sex).

RESULTS AND DISCUSSIONS

Out of the initial 977 tested samples, only 258 tested positive by real-time PCR. From the 258 eluates, only 68 had viral loads that allowed further testing using the classical PCR method, having CT values < 26.0. Seventy-five of them had CT values < 30 but greater than 26, while 115 had values above 30.

The samples included in the study were collected from a heterogeneous population of cats, primarily from the Bucharest municipality area. From all 977 samples tested for Feline Coronavirus only in 26.4% the virus was detected, which means that 258 samples were positive. Out of the 258 cats that tested positive for Coronavirus, 157 were males and 51 were females, which can be translated into percentages as 60.85% males and 39.15 females. In terms of breed distribution, the largest proportion was made up of European-breed cats, with 168 individuals, representing 65.11%. The British Shorthair breed accounted for 18.99%, with 49 cats diagnosed with the feline coronavirus. Additionally, 7 Persian cats (2.7%), 8 Scottish Fold cats (3.1%), and 6 Maine Coon cats (2.32%) were also diagnosed. The Norwegian Forest breed accounted for 1.55%, with 4 cats in the study. The Ragdoll breed was represented by 3 cats (1.62%), while the Sphynx, Siberian, and Devon Rex breeds each had 1 cat diagnosed (0.38% each) (Fig. 1).

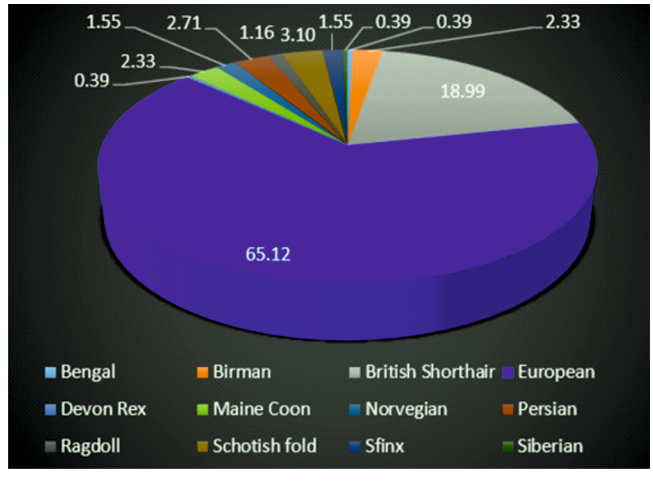

Fig. 1. Percentage distribution of breeds tested positive by real-time PCR

In terms of age category distribution, the most affected were cats aged between 1 and 5 years, representing 56.59% of the total positives. Young cats, under the age of one, were diagnosed positively in a number of 62, representing 24.03%. Mature cats, aged between 5 and 10 years, accounted for 8.14%, while senior cats (>10 years) also had a positive diagnosis for feline coronavirus in a proportion of 8.14% (Fig. 2).

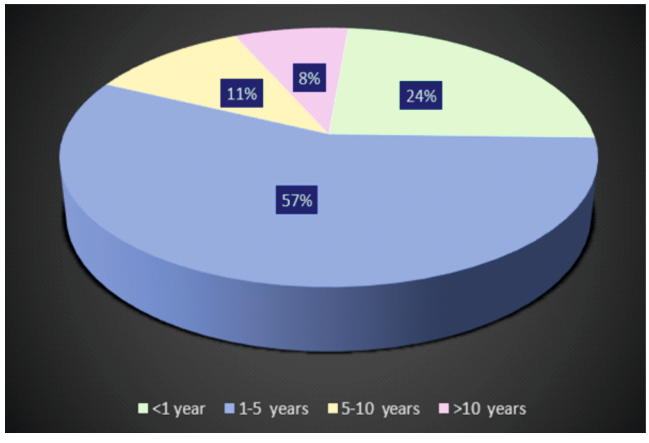

Fig. 2. Age category percentage distribution

CONCLUSIONS

Feline Infectious Peritonitis caused by Coronavirus is often suspected, but around a quarter are confirmed as positives. The viral loads are far from being high. Of the 258 positive samples, only 68 had viral loads that were high enough (CT values < 26) to allow further testing using the classical PCR method. This suggests that not all positive samples had significant viral loads, which could affect the ability to confirm infections with other methods. A large proportion (115 out of 258) had CT values above 30, indicating lower viral loads. Even so, the spread in the territory is high, given the pathogenicity characteristics. By observing the distribution of positive samples according to sex, we can conclude that males occupy a significantly higher percentage than females, which may be an indicator of a possible sex-related predisposition. Although the distribution of positive samples by breed is mostly dominated by the European breed, we cannot state with certainty that this breed is more predisposed than others, as we do not have clear data regarding the distribution of the breed in the territory. The European breed (Domestic Shorthair) was the most common among the infected cats, comprising 65.11% of the positive samples. British Shorthair cats were the second most affected breed at 18.99%. Other breeds, such as Persian, Scottish Fold, and Maine Coon, had a smaller representation in the infected group, with the Norwegian Forest, Ragdoll, Sphynx, Siberian, and Devon Rex breeds showing minimal involvement. This suggests that the European breed may have a higher exposure or susceptibility to the virus. But the European breed is the most commonly encountered among the tested cats, which may explain why this breed is predominant in the study results. Although there is a significant proportion of positive cases in less common breeds (such as Persian, Devon Rex, or Sphynx), these are much less widespread compared to the European breed, which is far more common in households. Therefore, no clear conclusion can be drawn that there is a breed predisposition based solely on the relative number of cases in a population of cats that is naturally much larger. Low Ct values found on this survey lead us to the idea that on many occasions the virus has undetectable levels even for the PCR method.

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