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Research Article / Araştırma Makalesi

Virological and Serological Investigation of Feline Coronavirus Infection in Cats

Kedilerde Feline Coronavirus Enfeksiyonunun Virolojik ve Serolojik Araştırılması

Ahmet TOPÇU[1], Yakup YILDIRIM[1*]

[1]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Virology, Burdur, Türkiye

Abstract: Feline coronavirus infection is an important viral disease affecting feline health. In this study, feline coronavirus infection (FCoV) in cats was investigated using virological and serological methods. For this purpose, blood and swap samples were taken from 60 cats aged six months or older with or without clinical signs in different races, sexes, ages and social environments, which were not vaccinated against the aforementioned infection and kept as pets at home. Collected blood samples were checked for FCoV antibodies by applying FCoV rapid test-antibody (Ab) and indirect Enzyme-Linked Immunosorbent Assay (ELISA) methods. Similarly, swap samples taken from the same cats were checked for the presence of FCoV antigen using the FCoV rapid test-antigen (Ag) method. Of the 60 cat blood serum samples analysed, 21 (35%) were determined to be seropositive by rapid test-Ab method and 41 (68.3%) by indirect ELISA method. The difference between the antibody positivity rates determined as a result of the indirect ELISA method and the rapid test-Ab method was found to be statistically significant (P<0.001). The presence of FCoV in 60 swap samples collected was investigated by rapid test-Ag method and 1 sample (1.7%) was found to be antigen positive. As a result of this research, the presence/prevalence of FCoV infection in owned cats was revealed both virologically and serologically. In addition, the indirect ELISA method was found to be more sensitive and reliable than the rapid test-Ab method in the serological diagnosis of FCoV infection.

Keywords: Antigen, Antibody, ELISA, Cat, Feline Coronavirus, Rapid test.

Öz: Feline coronavirus enfeksiyonu kedi sağlığını etkileyen önemli viral bir hastalıktır. Bu çalışmada, kedilerde feline coronavirus enfeksiyonunun varlığı (FCoV) virolojik ve serolojik yöntemler kullanılarak araştırıldı. Bu amaçla söz konusu enfeksiyona karşı aşılanmamış farklı ırk, cinsiyet, yaş ve sosyal çevrede bulunan klinik bulgu gösteren veya göstermeyen altı aylıktan büyük sahipli 60 kediden hem kan hem de dışkı örneklemesi yapıldı. Toplanan kan numunelerine FCoV antikor (Ab) hızlı test ve indirekt Enzyme Linked Immunosorbent Assay (ELISA) yöntemleri uygulanarak FCoV antikorları yönünden kontrol edildi. Benzer şekilde aynı kedilerden alınan dışkı numuneleri de FCoV antijen (Ag) hızlı test yöntemi kullanılarak FCoV antijen varlığı yönünden kontrol edildi. Örneklenen 60 kedi kan serumundan 21 adedinin (%35) rapid test-Ab yöntemi, 41 adedinin (%68,3) ise indirekt ELISA yöntemi ile seropozitif olduğu saptandı. İndirekt ELISA yöntemi ile rapid test-Ab yöntemi sonucunda belirlenen antikor pozitiflik oranları arasındaki farklılığın istatistiksel olarak önemli (P<0.001) olduğu tespit edildi. Toplanan 60 adet dışkı örneğinde de FCoV varlığı rapid test-Ag yöntemi ile araştırıldı ve bir numunede (%1,7) antijen pozitiflik belirlendi. Bu araştırmanın sonuçları, sahipli kedilerde FCoV enfeksiyonu prevalansının yüksek olduğunu ve enfeksiyonun serolojik tanısında indirekt ELISA yönteminin rapid test-Ab yöntemine göre çok daha duyarlı ve güvenilir bir yöntem olduğunu göstermektedir.

Anahtar Kelimeler: Antijen, Antikor, ELİSA, Kedi, Feline Coronavirus, Hızlı test.

*Corresponding author : Yakup YILDIRIM
e-mail : yyildirim@mehmetakif.edu.tr
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Introduction

Feline Coronavirus (FCoV), a common pathogen in cats, is classified in the *Coronaviridae* family. The pathogen has an enveloped, pleomorphic, ss (+) RNA genome. Most coronaviruses indicate tropism to mucosal epithelial cells of the intestinal and respiratory tract. Additionally, some coronavirus infections may lead to serositis,

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encephalitis, and hepatitis. Previous studies have identified two biotypes of FCOV: Feline Enteric Coronavirus (FECV) and Feline Infectious Peritonitis Virus (FIPV) (Pedersen, 1987; Vijaykrishna et al., 2007). The FECV, the apathogenic form of FCoV, is commonly isolated in the digestive tract of domestic and wild cats and may predispose these animals to some other pathogens. In infected animals, it has been reported that mutations in the nucleic acid of FECV lead to macrophage tropism, resulting in the formation of the FIPV biotype, which in transform causes feline infectious peritonitis (FIP), a systemic infection with a high mortality rate. FIP, which develops in cats with the effect of many unfavorable factors, can be seen in effusive (wet) and non-effusive (dry) clinical forms (Vennema et al., 1998; Lin et al., 2009).

Enteric coronavirus is transmitted to susceptible individuals by fecal-oral transmission. Infection in young animals is characterized by diarrhea lasting 3-4 days, while older animals are mostly asymptomatic. The causative agent has affinity for the epithelium of the large intestine, ileum, and rectum and can persist in these regions for up to 18 months. In cats persistently infected with FECV, mutations in the S protein of the virus have been suggested to enable the virus to evade the immune system (Herrewegh et al., 1997; Lappin, 2014). Although enteric coronaviruses are found in the bloodstream and lymph nodes, the pathogenesis of infection is limited to gastrointestinal symptoms (Vogel et al., 2010). The diagnosis of infection should be based on a combination of laboratory test results (serologic, virologic, biochemistry) and clinical symptoms.

In this study, it was aimed to determine the virological and serological presence of FCoV infection in nonvaccinated cats (owned/unowned) of different gender, age and social environment, with or without clinical signs, older than six months and to gather data about the prevalence of the disease. It was also aimed to compare the sensitivity of indirect ELISA and rapid test (Ab) methods used in this study in the serologic diagnosis of FCoV infection.

Materials and Methods

Ethical Approval

This research was conducted after the approval of Burdur Mehmet Akif Ersoy University Animal Testing Local Ethics Council (Approval Number: 26.08.2020-80/662)

Sampled Animals

In this study, Feline Coronavirus (FCoV) infection was studied virologically and serologically in owned cats of different genders, ages, and social environments, with or without clinical symptoms, who came to the clinics in Antalya and Burdur provinces. For this purpose, fecal and blood samples were collected from 60 cats (25 males + 35 females) with or without clinical symptoms and not vaccinated against the infection. The sampled cats were aged between 6 months and 15.5 years. Anamnesis information and clinical symptoms, if any, were recorded during collection.

Collection of Samples

Blood samples were collected from the *vena cephalica antebrachii* into sterile coagulated tubes and fecal samples were collected from the rectum into fecal sample containers and brought to the laboratory under cold storage conditions. Fecal samples were stored at -80°C until testing. After the blood samples were centrifuged at 5000 rpm for 15-20 minutes, the separated serum part was transferred to sterile stock tubes and stored at -80°C until the test phase.

Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

Serum samples obtained from blood samples collected from cats were analyzed for the presence of FCoV-specific antibodies (Ig) using a commercial indirect ELISA kit (FCoVCHECK Ab ELISA, Biopronix-Agrolabo, code 27224496,

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Torino-Italy) as described by manufacturer's protocol. The results were obtained quantitatively by measuring the optical density (OD) of each well of the plates spectrophotometrically at 450 nm in ELISA reader (Mindray MR-96A, Hamburg-Germany), and calculations were performed as described in the kit protocol.

FCoV Antibody (Ab) Rapid Test

A commercial Rapid FCoV Ab test kit (Asan Easy Test FCoV Ab, code no: 023351, Seoul, Republic of Korea) was used for the detection of FCoV-specific antibodies (Ig) in blood serum according to the manufacturer's procedure. This test is an immunochromatographic method for the rapid and quantitative detection of FCoV specific antibody in serum, plasma or whole blood of cats.

FCoV Antigen (Ag) Rapid Test

A commercial Rapid FCoV Ag test kit (Asan Easy Test FCoV Ag, code no: 23121, Seoul, Republic of Korea) was used for the detection of FCoV antigen in collected cat fecal samples. The test was performed according to the manufacturer's procedure and the results were evaluated.

Statistical Analysis

The statistical analysis of the data obtained in the study was performed using the SPSS 21 package program (IBM SPSS Software, USA). Chi-square (chi-square -$\chi 2$) test was used to determine the statistical significance of the differences in the positivity rates determined in different age groups in males and females as a result of the analysis of blood serum and fecal samples using ELISA and Rapid Test Ab/Ag. Furthermore, the statistical significance of differences in positive rates determined by rapid test-antibody and ELISA was examined by Chi-Square test. Data with a value of $P<0.05$ were considered significant.

Results

In the study, the seroprevalence rate of FCoV infection was 68.3% (41/60) in 60 cat blood sera analyzed by indirect ELISA diagnostic method and 35% (21/60) by FCoV Ab rapid test method. In the study, among the 60 fecal samples analyzed by FCoV antigen rapid test technique, antigen positivity (1.7%) was detected only in the sample of a 7-month-old female Tabby (sarman) cat. This cat had symptoms like effusive (wet) FIP and diarrhea. Blood serum sampled from this cat was also positive by both indirect ELISA and FCoV Ab rapid test.

When we observed the distribution of FCoV antibody positivity detected by ELISA method according to gender in the study, it was found that 64% (16/25) of male cats and 71.4% (25/35) of female cats were positive (Table 1).

In the study, antibody positivity determined by FCoV antibody rapid test method in cats was 40% (10/25) in male cats and 31.4% (11/35) in female cats (Table 2).

Table 1. Distribution of FCoV seropositivity detected by ELISA method according to gender.

Gender	Sample No.(n)	FCoV Ab			
		n (+)	%	n (-)	%
Male	25	16	%64	9	%36
Female	35	25	%71.4	10	%28.6
Total	**60**	**41**	**%68.3**	**19**	**%31.7**

Ab: Antibody

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Table 2. Distribution of FCoV seropositivity detected by FCoV antibody rapid test method according to gender.

Gender	Sample No. (n)	FCoV Ab			
		n (+)	%	n (-)	%
Male	25	10	%40	15	%60
Female	35	11	%31.4	24	%68.6
Total	**60**	**21**	**%35**	**39**	**%65**

Ab: Antibody

In the study, FCoV seropositivity detected in cats using indirect ELISA was 73.9% (17/23) in cats aged 6 months-1 year, 82.4% (14/17) in 2-year-old cats, 60% (6/10) in 3-year-old cats, 50% (1/2) in 4-year-old cats, 50% (2/4) in 5-year-old cats, and 25% (1/4) in cats aged 6 and older. (Table 3).

A total of 43.5% (10/23) in cats aged 6 months-1 year, 17.7% (3/17) in 2-year-old cats, 60% (6/10) in 3-year-old cats, 0% (0/2) in 4-year-old cats, 50% (2/4) in 5-year-old cats and none of cats aged 6 and over were detected FCoV seropositive according to age by rapid test method (Table 4).

Table 3. Distribution of FCoV seropositivity detected by ELISA according to age groups.

Age	Sample No. (n)	FCoV Ab			
		n (+)	%	n (-)	%
6 months-1 year	23	17	73.9	6	26.1
2	17	14	82.4	3	17.7
3	10	6	60	4	40
4	2	1	50	1	50
5	4	2	50	2	50
≥ 6	4	1	25	3	75
Total	**60**	**41**	**68.3**	**19**	**31.7**

Ab: Antibody

Table 4. Distribution of FCoV seropositivity detected by rapid test (Ab) according to age groups.

Age	Sample No. (n)	FCoV Ab			
		n (+)	%	n (-)	%
6 months-1 year	23	10	43.5	13	56.5
1-2	17	3	17.7	14	82.4
3	10	6	60	4	40
4	2	0	0	2	100
5	4	2	50	2	50
≥ 6	4	0	0	4	50
Total	**60**	**21**	**35**	**39**	**65**

Ab: Antibody

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Statistical Results

In the study, no statistically significant difference was found between males and females in seropositive-seronegative changes because of indirect ELISA analysis of blood serum samples taken from the animals ($\chi2$= 0.372; P=0.542). Similarly, there was no significant difference between the seropositive values determined in males and females in the Rapid antibody test in blood samples ($\chi2$= 0.471; P>0.05) and Rapid antigen test in feces ($\chi2$= 0.726; P>0.05). Again, the distribution of both antibody and antigen-positive rates in general age groups was statistically insignificant (P>0.05). However, it was determined that there was a significant (P<0.05) difference in the statistical evaluation of seropositivity detected by ELISA between 2 years and younger and 3 years and older ($\chi2$= 4.660; P=0.031). In the statistical analysis of the seropositive values in the Rapid antibody test and ELISA results, which form the basis of our study, a statistically significant P<0.001 difference was found between the groups ($\chi2$= 13.348).

Discussion

Coronaviruses are pathogens that cause different levels of infection in humans and animals. Many viruses in this family have high mutation ability and the capability to transmit between species. For this reason, it is very difficult to apply protection and control methods against CoV types. FCoV infections are a highly contagious disease common in wild and domestic cats worldwide. Fighting the infection becomes very difficult because the disease is very common, especially in places where domestic cats are kept together (cats on breeding farms, cat shelters, etc.) and can be detected in cat of all age groups.

Due to the high mortality rate observed in the FIP form of the disease, it is important not to waste time in diagnosis and treatment. For this purpose, researchers are working hard to develop new diagnostic methods and to improve their reliability. In addition to clinical symptoms, virological, serological and molecular techniques and histopathological examination are performed to accurately diagnose the infection (Tasker, 2018; Zhao et al., 2019). In addition to these methods, rapid immunoassay kits have been developed to detect FCoV antibodies and antigens. However, with the current research results on the reliability of the tests, the question marks in mind have started to increase.

In FCoV seroprevalence studies conducted worldwide, proportional changes have been detected between countries. In studies using the ELISA method (Bell et al., 2006; Pratelli, 2008; Tharaguchi et al., 2012; Suba et al., 2016; Mūrniece et al., 2021) the seroprevalence of the disease has been reported between 34% and 89.5%. Studies on the seroprevalence of FCoV in Turkey (Pratelli et al., 2009; İleri, 2013; Oğuzoğlu et al., 2010) reported rates between 26% and 69.8%. In our study, the seroprevalence of FCoV infection was 68.3% by indirect ELISA method and 35% by rapid test (Ab) method. These results are in parallel with the results of previous studies.

In this study, it was determined that the distribution of antibody positivity according to gender was not statistically significant. This result is consistent with other studies in the literature (Bell et al., 2006; Oğuzoğlu et al., 2010; Tharaguchi et al., 2012; Mūrniece et al., 2021). As a result of the rapid test (Ag) conducted on fecal samples in our study, the FCoV antigen rate in our sample group was determined as 1.7% (1/60). This result is lower than the prevalence values determined in previous antigenic studies on FCoV (Can-Şahna et al., 2007; Loock et al., 2021; Vojtkovska et al., 2022). This was thought to be since the study was conducted with a limited number of samples and the sensitivity of the test method used was low. However, the limited number of studies in this field makes our findings important. When the seropositivity rates of ELISA and rapid test (Ab), which is another perspective of our study are compared there is a significant difference detected that supports previous research.

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In a study by Addie et al. on the effectiveness of IFA, ELISA and rapid test kits in the diagnosis of FCoV, they found positivity rates of 100%, 100% and 64.1-84.6%, respectively. In this study, samples that had not been previously tested for FCoV and included in the study by random sampling method were evaluated and sensitivity rates were found to be 35% in Rapid Ab and 68.3% in indirect ELISA method. In light of these data, it was revealed that the difference between positive result rates was statistically significant. Accordingly, it has been concluded that the ELISA method provides more effective and highly reliable results in laboratory diagnosis than rapid antibody tests.

Although the cats were sampled for the study were in different age groups, it was found that there was a statistically significant difference in antibody positivity rates between cats between 6 months and 2 years of age and cats in other age groups (3≥). This result is in line with other studies (Cave et al., 2004; Hartmann, 2005; İleri, 2013).

This may be explained by the fact that the immune system in young cats is not fully developed or is susceptible to diseases. Therefore, regular FCoV screening in young cats will be useful for the early diagnosis of the disease. Although the animals used in our study were owned, considering that they are in constant contact with the environment and other cats and that cat owners can adopt new cats from unknown sources, our seroprevalence value is parallel to the results of research conducted on environments where more than one cat lives together and on stray cats (Bell et al., 2006; Pratelli et al., 2009)

As a result, our study revealed that the ELISA method is more sensitive than rapid test kits (Ab) for detecting FCoV seropositive cats. Preferring high-sensitivity methods such as the ELISA method in the diagnosis of suspicious cases will allow the veterinarian to initiate the correct treatment protocol in a timely manner and will prevent the patient from going through too much stress by saving him from many different procedures whose results are not clear. As FCoV infection is widespread in domestic cats and poses a risk to feline health, it is concluded that it would be beneficial to give importance to protection and control measures against infection. In this context, vaccination against the infection in question will be beneficial.

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