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Epizootological Features of Coronavirus Infection in Cats

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Abstract. The relevance of the study of coronavirus infection in animals is conditioned upon the lack of sufficient information about the mechanisms of development of this disease, imperfect methods of diagnosis and treatment, and, most importantly, almost 100% of their mortality. The purpose of this study was to identify the age, breed, seasonal, and sexual characteristics of cats' predisposition to coronavirus infection and the development of infectious peritonitis. The paper presents the results of epizoothogolic features of the spread of coronavirus infection among animals of this species and feline infectious peritonitis during 2020-2022 based on the veterinary clinic "White Wolf" (city of Kyiv). During this period, 483 samples were examined from cats with symptoms of coronavirus infection. From these samples, using immunochromatographic analysis, a virus of the *Coronaviridae* family was detected in 399 animals, and infectious peritonitis was established in 63 animals. This paper highlights the results of a study of the age, breed, and sexual predisposition of cats to coronavirus infection and the development of infectious peritonitis. It was found that cats of any age are susceptible to coronavirus infection, while infectious peritonitis develops in animals aged from 2 months to 3 years. Coronavirus infection was most frequently found in mixed breed cats and British shorthair cats, infectious peritonitis – in Burmese and Bengal breeds. The study also summarised data on the seasonal manifestation of coronavirus infection and infectious peritonitis of cats. Based on the results of epizootological analysis, a nosological profile of infectious diseases was formed in animals of this species that had similar clinical symptoms of coronavirus infection and infectious peritonitis. Eight infections are presented, of which the most frequently recorded diseases were caused by viruses of the *Herpesviridae, Caliciviridae, and Parvoviridae* families. The results of this study provide new information about the epizootological features of the manifestation and development of coronavirus infection in cats, which allows not only improving the available diagnostic methods, but also developing new ones

Keywords: breed, sensitivity, seasonality, epizootic process, real-time PCR, test systems

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Introduction

Coronavirus infection in cats is a contagious disease of wild and domestic animals and is widespread all over the world. Thus, according to various sources, from 73% to 90% of cats are infected with coronavirus. Only 2-13% of these animals, which are carriers of the intestinal type of coronavirus, can develop infectious peritonitis. The latter is manifested clinically by fibrinous polyserositis and accumulation of a significant volume of exudate and is also characterised by high mortality [1; 2].

The feline coronavirus has two different genotypes, called feline intestinal coronavirus (FCoV) and feline infectious peritonitis virus (FIPV), which show different clinical symptoms. Feline intestinal coronavirus mainly infects cats by fecal-oral route and causes mild and transient gastroenteritis, frequently leading to the development of asymptomatic infections. The feline infectious peritonitis virus, on the contrary, occurs due to a mutation of the feline intestinal coronavirus and is observed in a small percentage of infected animals [1].

Feline infectious peritonitis today is still one of the most studied infectious diseases. This is because the mechanism of development of the disease, diagnosis, and treatment are understudied, and almost 100% mortality encourages further study of this infection [1]. Coronavirus is one of the most common pathogens of cat diseases. Infection of cats with coronavirus is quite common, usually characterised by the development of mild intestinal infections, the main sign of which is diarrhoea. Feline coronavirus (FCoV) is a contagious disease of this animal species and occurs worldwide [2].

Notably, the epizootological characteristics of coronavirus infection in cats, including infectious peritonitis of cats, are understudied, which creates the need to analyse statistical data on the results of clinical and laboratory studies of these animals.

The relevance of this subject is indisputable, and therefore this study is primarily aimed at clarifying and generalising data on the epizootological features of the spread of coronavirus infection and infectious peritonitis among cats. This will provide an epizootological insight into the situation regarding these diseases and possible solutions to prevent the development of these diseases.

The purpose of this study was to identify the predisposition of cats to coronavirus infection and the development of infectious peritonitis depending on age, breed, time of year, and sex.

Literature Review

Coronaviruses are RNA-positive single-stranded viruses belonging to the *Coronaviridae* family of the *Nidovirales* order. They are divided into four genera: Alpha-, Beta, Gamma-, and Delta-coronaviruses. Gamma and Delta coronaviruses mainly affect birds. Alpha- and Beta- coronaviruses affect only mammals. Coronaviruses have the largest genome among RNA viruses, the size of which ranges from 26 to 32 kb [2].

Upon study, coronaviruses show a high mutation rate during replication and therefore exist as clusters of genetically diverse populations. Feline coronavirus infection (FCoV) has been found to be widespread all over the world, with a few isolated islands [3].

There are two genotypes of cat coronavirus: type 1, which represents the vast majority of field isolates, and type 2, which occurs due to recombination between the feline coronavirus type 1 and the canine coronavirus. The two serotypes differ primarily in their transmembrane adhesive (S) glycoprotein [3-5].

Infection of cats with the intestinal type of coronavirus reaches about 35% of the population of these pets. Antibodies to FCoV were detected, which indicates virus transmission in cats. Thus, in households with one cat, the prevalence of intestinal coronavirus type decreases to 21%, but, accordingly, in households with many cats, the prevalence can exceed 90%. Most cases of intestinal coronavirus in cats are transient infections, and only a small percentage of animals become resistant “carriers” of coronavirus [4].

The solitary lifestyle of the ancestors of domestic cats led to the emergence of a variety of virus transmission pathways and stimulated the appearance of latent, chronic and/or asymptomatic infections, which contributed to a decrease in the level of immune response of the sick animal's body, and eventually led to an increase in the population of the pathogen carriers [5].

In their studies, C. Li et al. (2019) established the prevalence of feline intestinal coronavirus [6]. According to their data, FCoV is common in most cat populations. Moreover, the infection rate exceeds 20%: in northern China – 80%; southern China – 73.1%; southwest China – 80.4%; eastern China – 74.6%; Portugal – 47.5%; Germany – 76.5%; Malaysia – 84%; southern Italy – 80%; Japan – 37%.

L.A. McKay et al. (2020) found that feline coronavirus type 1 was the only serotype found in fecal and tissue samples in North American and European cat populations. It has already been established that 46% of cats are carriers of feline coronavirus [7].

W. Zhang et al. (2014) examined the feces of 25 cats from the shelter for the presence of various pathogens of infectious diseases [8]. Therewith, eight viral families were identified: *Astroviridae, Coronaviridae, Parvoviridae, Circoviridae, Herpesviridae, Anelloviridae, Caliciviridae* and *Picobirnaviridae*. Among the coronaviruses, feline type 1 coronavirus was identified in 15 fecal samples under study.

There are two forms of manifestation of coronavirus infection in cats: feline infectious peritonitis and the actual coronavirus infection of cats, which affects the intestines of animals [9].

Infectious feline peritonitis is an infection that is common among wild and domestic cats and is described by a high degree of mortality. It is manifested by the development of fibrinous and granulomatous polyserositis, the accumulation of protein-rich serous effusion in body cavities, and/or granulomatous inflammatory lesions of organs. The causative agent of feline infectious peritonitis is the feline coronavirus, which is widespread all over the world. The proportion of FCoV-infected cats that can develop feline infectious peritonitis is estimated at 5-12% [1; 9].

The feline infectious peritonitis virus, unlike the intestinal coronavirus, is not contagious and is not transmitted by oral-fecal route, but occurs as a result of a mutation from avirulent FCoV in 11-13% of infected cats, which leads to the development of a serious disease – infectious feline peritonitis, which is lethal [10].

Feline infectious peritonitis (FIP) can occur in animals of any age but is more common in cats younger than three years and especially between the ages of 4 and 16 weeks. Feline infectious peritonitis is observed in kennels (breeding cats), animal shelters, and places with a high concentration of animals per 1 m^2. Typically for an enzootic infection, the incidence of feline infectious peritonitis can vary widely over time. Mortality from infectious peritonitis in cats is very high, especially after the onset of clinical symptoms, although some animals can live with this disease for several weeks, months, or, rarely, years [11; 12].

Risk factors contributing to the development of infectious peritonitis in cats include those that affect the rate of transmission and replication of the virus in the animal's body, including young age, reduced immune response of the body to the causative agent of the disease, physiological stress, viral load in the environment, group habitat of animals and genetic predisposition [9].

Y. Yin et al. (2021) found that most cats suspected of infectious peritonitis belonged to young animals (under 3 years of age) and intact males [1]. They also determined that the effusive form of infectious peritonitis was observed more frequently, i.e., in 85.8% of animals with diagnosed feline infectious peritonitis.

According to S.J. Yen and H.W. Chen (2021) infectious peritonitis was detected in 47.7% of purebred animals and 47.1% in the mongrel cat group [13]. Purebred cats included the following breeds: American Shorthair, British Shorthair, Scottish Fold, Russian Blue, Chinchilla, Munchkin, and Ragdoll. The age-related predisposition to feline infectious peritonitis was also established. Therewith, the authors of this study found that the highest positive result for feline infectious peritonitis was obtained in the group of animals aged from 0 to 24 months – 67.4%, and the lowest – in the group of cats older than 73 months – 15.6%.

Q. Zhou et al. (2021) found that infectious feline peritonitis is more common in males (48.4%) and 44.2% among females. The author also determined the prevalence of feline infectious peritonitis relative to the density of animal habitat. Thus, among cats raised in groups, the incidence of infectious peritonitis was 58.1%, while among cats raised alone – 37.2% [14].

Based on the results of the study by Y. Yin et al. (2021), of the 59 cats with infectious peritonitis under study, 26% of the sick cats were female (11 animals) and 74% were male (32 animals). Of the 59 animals under study, 47 belonged to the group of young animals – from 2 months to 2 years [15].

Materials and Methods

The material for the research was cats with coronavirus infection during 2020-2022. The study was carried out based on the Department of Epizootology, Microbiology and Virology of the National University of Life and Environmental Sciences of Ukraine and the veterinary clinic "White Wolf" (Kyiv). The analysis of the epizootic situation of coronavirus infection in cats was carried out by analysing the data of the journal of registration of sick animals for the above period, considering the data of anamnesis and clinical and laboratory studies. The specific features of the spread of coronavirus infection in cats and infectious peritonitis in relation to the age, time of year, sex, and breed of sick animals were determined. The prevalence of various forms of infectious peritonitis in cats was determined. The nosological profile of infectious pathology circulating among cats that were admitted to the above-mentioned clinic for comprehensive studies was established.

To identify the prevalence of various feline infectious diseases, rapid blood tests of animals were performed using test systems based on three-phase immunochromatographic analysis (ICA). The authors of this paper used test systems from VetExpert.

Laboratory tests of blood samples and effusion from the pleural and abdominal cavities were carried out based on the laboratory of the veterinary clinic "White Wolf" and the veterinary laboratory "BioSoft" (Kyiv). The sampling of exudate from the chest and abdominal cavities of cats with an effusive form of infectious peritonitis was carried out under the control of an ultrasound diagnostic device (USD device).

Feline Corona Virus Antigen (FCoV Ag) Test of the Asan Pharm company was used to study fecal samples of sick cats. To confirm the diagnosis of infectious peritonitis in cats, polymerase chain reaction (PCR) was performed with samples of exudate from the thoracic or abdominal cavities in the case of an effusive form of infectious peritonitis in cats. As an auxiliary diagnostic method, an enzyme-linked immunosorbent assay (Elisa) was used to identify virus-specific antibodies.

In the dry form of infectious peritonitis in cats, the antibody titre in the blood serum was also identified using Elisa. For this, venous blood was taken and centrifuged for 10 minutes at 3,000 rpm. Quantitative PCR (RealPCR Test) was used to establish the diagnosis. The principle of operation of this test is that specific short fluorescent hydrolysis probes either detect two separate nucleoside mutations in feline coronavirus (FeCV) and FIPV or set the nucleotide sequence in wild-type FeCV at a different fluorescence wavelength.

For the study, a maximum of 1 cm^3 of native EDTA-stabilised blood was taken, centrifuged for 10 minutes at 2,500 rpm. Plasma was separated from blood cells and stored at -20°C. One volume of PBS was added to the blood cells. Total RNA was isolated according to the Total Quick RNA Blood Kit Protocol (Talent) [16].

For real-time PCR, oligonucleoside primers that respond to the M gene sequence (primer 212) of the FeCV genome were used with a primer targeting the FCoV genome leader sequence (primer 1179).

The reaction mixture was placed in a thermal cycler (Biozym). The temperature cycle protocol consisted of 10 minutes of incubation at 95 °C, followed by 30 cycles of 1 minute of denaturation at 95 °C, 1 minute of primer annealing at 62 °C, and 1 minute of primer elongation at 72 °C. After 30 cycles, the reaction mixture was kept for 10 minutes at 72 °C, followed by cooling to a temperature of 4 °C.

N.C. Pedersen [11] et al. [7; 13] report that a definitive diagnosis of feline infectious peritonitis can be made by immunohistochemical examination. However, the authors of the paper did not use this method due to the lack of proper equipment in Ukraine.

Digital data were processed biometrically, applying methods of statistical analysis using computer programs Statistica 6.0 and Microsoft Excel 2019.

Results and Discussion

The main clinical symptoms observed in feline coronavirus infection and infectious peritonitis include vomiting, diarrhoea, weight loss, lethargy, refusal of food, accumulation of exudate in the chest and abdominal cavities.

Of all the samples under study using test systems, the feline coronavirus antigen was detected in 399 samples, which is a percentage of 82.6% of animals (399/483). Therewith, feline infectious peritonitis was detected only in 63 animals out of 483, which is 13%.

It was found that among cats registered in the veterinary clinic "White Wolf" there were seven infectious diseases of cats, namely panleukopenia, coronavirus infection, herpesvirus infection, calicivirus infection, feline infectious peritonitis, feline immunodeficiency virus and feline leukaemia virus (Table 1).

Table 1. Nosological profile of feline infectious diseases detected in 2020-2022, n = 483

Diseases	% of sick animals	Number of sick animals
Panleukopenia	23.0	111
Coronavirus infection	82.6	399
Herpesvirus infection	31.0	150
Calicivirus infection	14.2	69
Feline infectious peritonitis	13.0	63
Feline immunodeficiency virus	2.9	14
Feline leukaemia virus	1.2	6

Laboratory tests revealed the pathogens of the above infections: panleukopenia – 23.0%; feline coronavirus infection – 82.6%; herpesvirus infection – 31.0%; feline calicivirus infection – 14.2%; feline infectious peritonitis – 13.0%; feline immunodeficiency virus – 2.9%; feline leukemia virus – 1.2%.

Infectious diseases such as panleukopenia, coronavirus infection, and herpesvirus infection were more frequently reported. Notably, the feline coronavirus was registered simultaneously with other diseases. Thus, out of 111 cats with panleukopenia, 57 were found to have feline coronavirus. This indicates that coronavirus infection in cats can occur with other diseases with a decrease in immunity.

As a result of epizootological examination of sick cats with clinical symptoms of infectious peritonitis (anorexia, weight loss, accumulation of exudate in the chest/abdominal cavity, uveitis, nervous system damage), 13.0% were found, of which males – 54.0% (34/63), females – 46.0% (29/63) (Fig. 1).

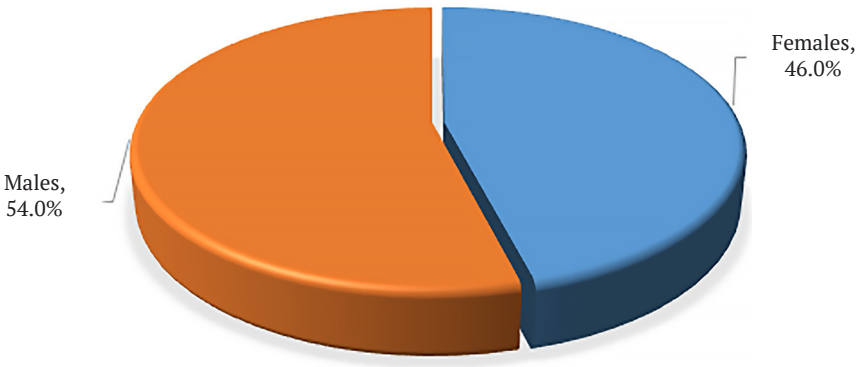

Figure 1. Predisposition to feline infectious peritonitis depending on gender

From this, it can be concluded that both male and female cats are equally susceptible to feline infectious peritonitis. Authors N.C Pedersen, S. Felten claim that intact males are more susceptible to feline infectious peritonitis [10; 11]. N.C. Pedersen (2014) [11] found that male cats, especially intact males, are not directly at risk of developing feline infectious peritonitis, as other authors claim [13].

Age-related features of the spread of feline coronavirus infection and feline infectious peritonitis were established. Among the 399 cats under study, the largest proportion of patients with feline coronavirus infection was observed in the group of animals from 1 to 2 years (26%) and in the group of 2-5 years (28%) (Fig. 2).

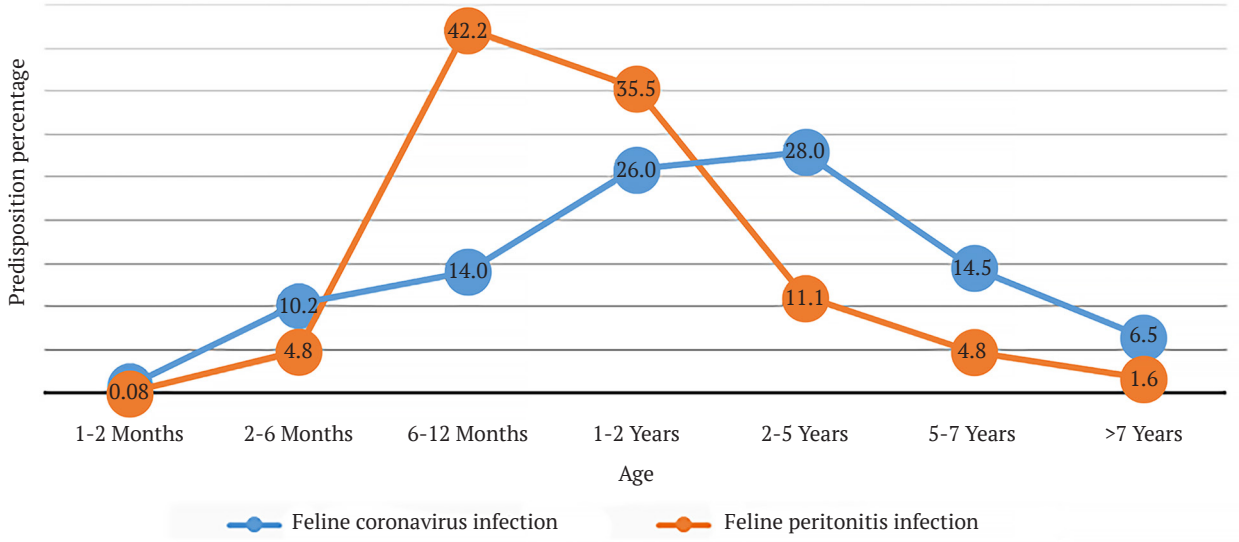

Figure 2. Predisposition to feline infectious peritonitis depending on gender

It was found that animals of geriatric age (older than 7 years) also get sick. 25 such cases were registered, which was 6.5%. Among kittens of the first two months of life, only 3 cases of coronavirus infection were detected, which may be due to infection from the mother. The above results are consistent with the literature data [7; 13].

Among the 63 cats studied with infectious peritonitis, it was determined that the disease is most often registered in young animals (Fig. 2). Thus, the largest proportion of sick cats was found at the age of 6 to 12 months (41.2%) and in the group of 1-2 years (35.5%). There was also 1 case of feline infectious peritonitis in the geriatric group of animals (older than 7 years), which is 1.6%.

Analysis of data from the journal of registration of sick animals for 2020-2022 allowed establishing and determining the seasonal manifestation of feline coronavirus infection (Fig. 3). According to the results obtained, the largest number of cases of feline coronavirus infection was registered in summer (26.8%) and spring (32%), which is confirmed by literature data [12].

Figure 3. Features of seasonal manifestation of feline coronavirus infection

Analysis of the seasonal spread of feline infectious peritonitis showed that at each time of the year, the number of patients with infectious peritonitis in cats had approximately the same number – 20-30%. According to the research of E.N. Barker and S. Tasker (2020) [3] and N.C. Pedersen et al. (2015) [12] feline infectious peritonitis is also seasonally absent.

The breed predisposition to feline coronavirus infection and feline infectious peritonitis was determined. Among 399 positive samples for cat coronavirus infection, mongrel cats (31.3%), Bengal cats (14.8%) and British shorthair cats (17.0%) were found to be the most susceptible to infection (Fig. 4).

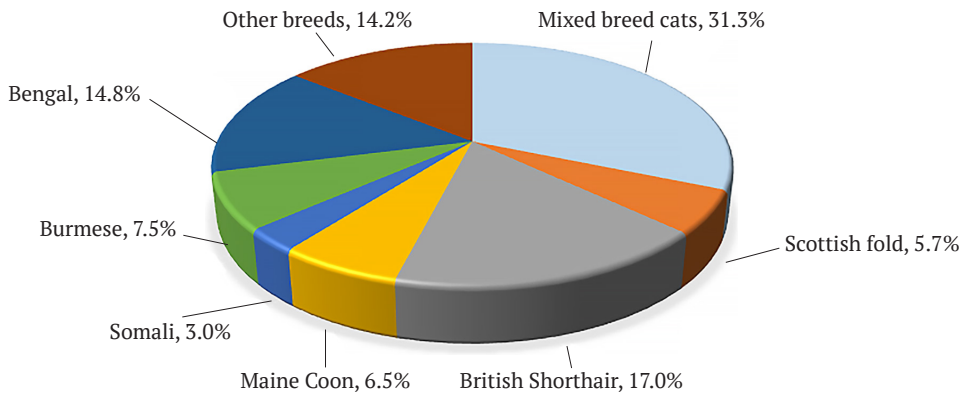

Figure 4. Percentage of breed predisposition of cats to feline coronavirus infection

According to research by S. Felten and K. Hartmann (2019) [10] and N.C. Pedersen (2014) [11] thought that mixed breed cats were more resistant to coronavirus infection, but most authors (Y. Yin, T. Li, C. Wang) [1] are against such a definition. Feline infectious peritonitis was diagnosed in 63 animals. When determining the breed predisposition to this disease, it was found that the largest percentage of sick animals is observed among Burmese cats (33.3%) and mixed breed cats (28.5%) (Fig. 5). Notably, many cases of feline infectious peritonitis were detected in the Bengal breed (13 out of 63).

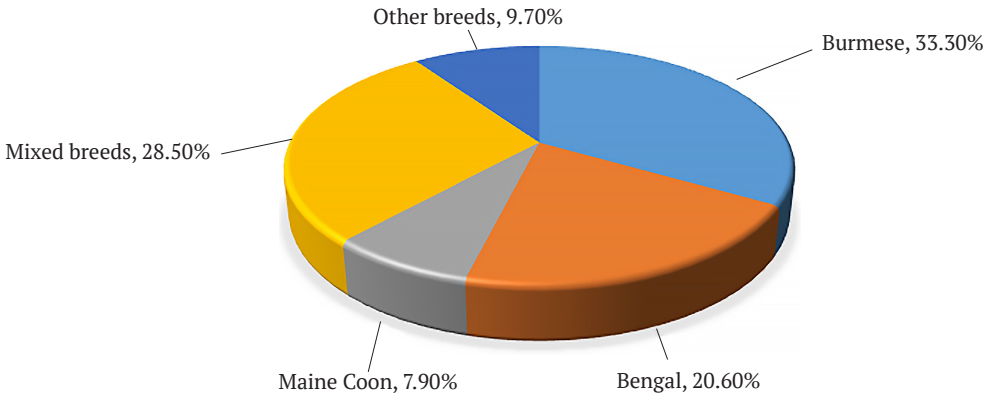

Figure 5. Percentage of breed predisposition of cats to feline infectious peritonitis

Among the analysed literature data, there is no reliable information about the breed predisposition to feline infectious peritonitis. The authors consider these data to be relative due to the diversity of populations in different cities and countries. However, to date, the predisposition to feline infectious peritonitis in Burmese cats has been determined, and the genetic component has been determined by GWAS (genome-wide association studies) [17].

Furthermore, data on the prevalence of forms of feline infectious peritonitis were analysed (Fig. 6). It is known that feline infectious peritonitis occurs in three forms – eufused (wet), dry, and mixed.

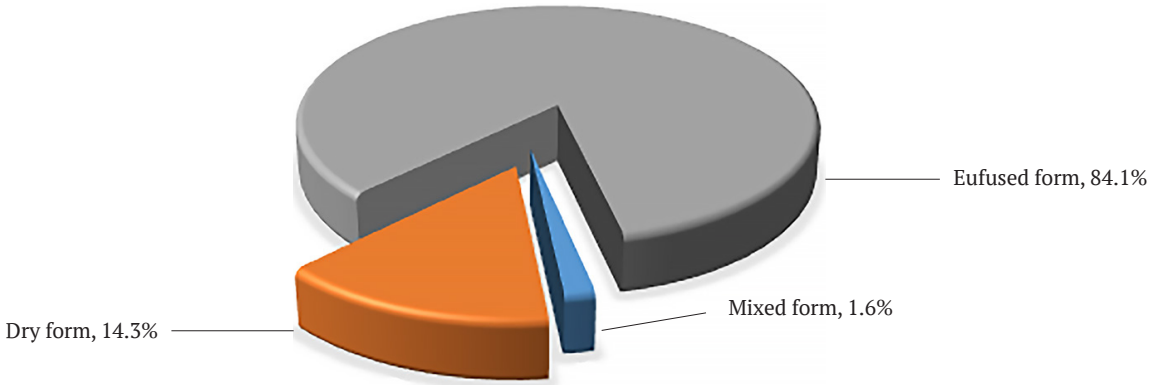

Figure 6. Percentage of breed predisposition of cats to feline infectious peritonitis

Of the 63 reported cases of feline infectious peritonitis, eufusion was detected in 84.1% (53 out of 63), dry – in 14.3% (9 out of 63). One cat had a mixed form of infectious feline peritonitis (1.6%). In this case, the accumulation of exudate in the abdominal cavity, the development of uveitis and keratitis were observed. Y. Yin (2021) et al. found that the eufusion form of feline infectious peritonitis is diagnosed more frequently, i.e., in 85.8% of animals with suspected feline infectious peritonitis [1].

In cats with a dry form of infectious peritonitis, the titre of antibodies to coronavirus was determined. Therewith, an enzyme-linked immunosorbent assay was used to determine the titre of Ig G antibodies. Nine blood sera from cats with a dry form of feline infectious peritonitis were examined in the laboratory.

Thus, it was found that in seven cats with a dry form of feline infectious peritonitis, the antibody titre corresponded to >1:160, in one animal – 1:50, and in another animal <1:20 (Fig. 7). This means that in cats with feline infectious peritonitis, the antibody titre to infection may be low. Since the titre of antibodies based on the clinical symptoms of the disease and a positive PCR test can give a negative result, it is inappropriate to use only ELISA for its diagnosis. N.C. Pedersen (2014) found that the antibody titre in cats with an effusive or dry form of feline infectious peritonitis in the late stages of the disease may be low, but this does not exclude the presence of the pathogen in the animal's body [11].

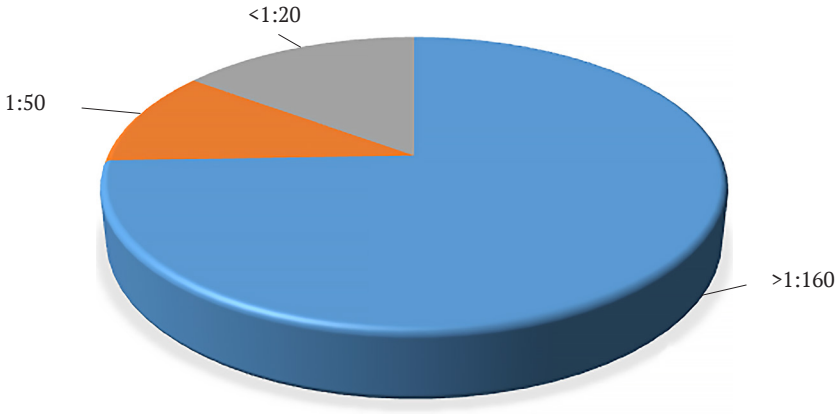

Figure 7. Titre of Ig G antibodies to coronavirus in cats with a dry form of feline infectious peritonitis

It was found that of the three animals under study who had symptoms of rhinitis, one cat was diagnosed with infectious peritonitis by PCR. Therewith, catarrhal discharge from the nasal passages and loss of the sense of smell were noted. E.N. Barker and S. Tasker (2020) [3] proved the possibility of developing rhinitis and conjunctivitis, which are associated with the development of feline infectious peritonitis, which confirms the reported case.

Conclusions

Studies indicate that the most sensitive breeds to feline coronavirus infection are British Shorthair and Bengal, as well as mestizo cats. Burmese and Bengal cat breeds are most susceptible to feline infectious peritonitis.

Along with this, it was found that gender and age do not affect the prevalence of feline coronavirus infection. The risk of feline infectious peritonitis is higher in kittens and animals aged 2 months to 3 years.

It was established that feline coronavirus infection can occur combined with other feline infectious diseases, especially with a complication of feline panleukopenia. At the same time, the coronavirus can be detected in clinically healthy animals that are carriers of the virus.

It was found that 84.1% of cats have a euphusial form of feline infectious peritonitis. One animal showed symptoms of rhinitis and conjunctivitis, which is a possible manifestation of the dry form of feline infectious peritonitis. This disease is non-infectious and occurs because of a mutation of the intestinal type of coronavirus in susceptible animals. Therefore, not all individuals with feline coronavirus infection are patients with feline infectious peritonitis.

Further studies should be aimed at investigating the features of the course, the main clinical symptoms of feline infectious peritonitis. Furthermore, it is necessary to identify the key diagnostic deviations in standard studies (general blood test, biochemical blood test), to establish new areas in the complex diagnosis of feline infectious peritonitis. A prominent issue is the determination of effective ways to diagnose feline infectious peritonitis, as well as the improvement of treatment measures for this disease.

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Епізоотологічні особливості коронавірусної інфекції у котів

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Анотація. Актуальність дослідження коронавірусної інфекції в тварин зумовлена відсутністю достатньої інформації щодо механізмів розвитку цього захворювання, недосконалістю методів діагностики та лікування і, найголовніше, майже 100 % їх летальністю. Метою цієї роботи було визначення вікових, породних, сезонних та статевих особливостей схильності котів до коронавірусної інфекції та розвитку інфекційного перитоніту. У статті наведені результати епізоотоголічних особливостей поширення коронавірусної інфекції серед тварин цього виду та інфекційного перитоніту котів упродовж 2020–2022 років на базі ветеринарної клініки «Білий Вовк» (м. Київ). За цей період було досліджено 483 проби від котів з симптомами коронавірусної інфекції. З цих проб за допомогою імунохроматографічного аналізу у 399 тварин було виявлено вірус родини *Coronaviridae*, а у 63 – встановлено інфекційний перитоніт. У роботі висвітлено результати дослідження вікової, породної та статевої схильності котів до коронавірусної інфекції та розвитку інфекційного перитоніту. Встановлено, що до коронавірусної інфекції є схильними коти будь-якого віку, тоді ж як інфекційний перитоніт розвивається у тварин віком від 2 місяців до 3 років. Коронавірусну інфекцію найчастіше реєстрували у котів-метисів та британських короткошерстних, інфекційний перитоніт – у бурманської та бенгальської їх порід. У статті також узагальнено дані сезонного прояву коронавірусної інфекції та інфекційного перитоніту котів. За результатами епізоотологічного аналізу було сформовано нозологічний профіль інфекційних захворювань у тварин цього виду, які мали схожі клінічні симптоми коронавірусної інфекції та інфекційного перитоніту. Представлено вісім інфекцій, з яких найчастіше реєстрували захворювання, спричинені вірусами родин *Herpesviridae, Caliciviridae* і *Parvoviridae*. Результати цієї роботи вносять нові відомості щодо епізоотологічних особливостей прояву та розвитку коронавірусної інфекції в котів, що дасть можливість не тільки удосконалювати існуючі методи діагностики, а й розробляти нові

Ключові слова: порода, чутливість, сезонність, епізоотичний процес, ПЛР в реальному часі, тест-системи