

Comparison of the sensitivity of rapid tests FCoV Ab (Vet Expert) and PCR in the diagnosis of feline infectious peritonitis (FIP) in cats with the effusive form of the disease

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Summary

The aim of the study was to determine the usefulness of FCoV Ab rapid serological tests in the diagnosis of the effusive form of FIP in cats. The cats included in the study were divided into two groups. The study group consisted of 40 cats with a strain of FCoV causing FIP (the presence of the M1058L mutation) in the abdominal fluid determined using PCR. The control group consisted of 15 cats with ascites caused by factors other than FCoV infection. Serological examination demonstrated the presence of antibodies to feline coronavirus in 28 out of 40 samples of the fluid collected from animals included in the study group, which constituted 70.0% of the tested samples. No antibodies to coronavirus were identified in any of the peritoneal fluid samples collected from the cats included in the control group using rapid immunochromatographic tests. The results obtained in our own studies demonstrated that the serological test ensured very high probability, especially in the detection of infected animals, as well as, although with a slightly lower probability, in the exclusion of the presence of FIP virus infection in the samples of fluid collected from the peritoneal cavity.

Keywords: cats, FIP, molecular test, serological test

Feline infectious peritonitis (FIP) is a fatal disease caused by a mutant feline coronavirus. The disease is more prevalent in free-ranging cats and in animals bred in catteries. FIP is primarily caused by the interactions between the virus and the host's immune system, which lead to vasculitis.

Feline infectious peritonitis virus belongs to the family Coronaviridae in the order *Nidovirales*. The virus is large and spherical, and its genome is made of a 27-32 bp long RNA molecule (1, 10). We distinguish 2 biotypes of feline coronavirus that differ in their pathogenicity: enteric and FIP-causing (9). In contrast to the widespread enteric biotype, the biotype that causes FIP is less common. In more than 95% of cases, the virulent coronavirus is characterised by

two mutation positions: 23531 and 23537 in the gene encoding the surface glycoprotein (S). The mutation causes the replacement of methionine with leucine at position 1058 in the amino acid sequence (mutation M1058L) and alanine with serine at position 1060 in the protein (mutation S1060A). These mutations are considered to be responsible for genotype change and increased viral tropism for macrophages (2, 15).

Two forms of FIP are distinguished as: effusive (wet), in the course of which fluid accumulates in the body cavities, and non-effusive (dry), characterized by the formation of granulomas in internal organs. The function of T cells, playing a key role in preventing disease progression, determines the form of the disease that develops in an infected cat. In animals

with immunosuppression and T cell deficiency, the coronavirus replicates extensively in monocytes. These cats experience viremia, facilitating the spread of the virus throughout the body. As monocytes migrate from the capillary veins to various tissues (including serous membranes), they release the virus and cytokines, which cause inflammation. This results in the formation of granulomas (pyogranulomas) in various organs and tissues – ranging from very small to large tumours (the non-effusive form of FIP). If granulomas are accompanied by the accumulation of an exudate in the body cavities, this condition is referred to as the effusive form of the disease (3, 14).

The clinical symptoms accompanying FIP are very diverse and depend on the form of the disease. Both forms of FIP begin with general symptoms, such as apathy, decreased appetite and weight loss, as well as fever. The infected cats may suffer from diarrhoea, vomiting, and sometimes jaundice (1, 3, 8, 14). Ascites is the most predominant symptom in the effusive form of the disease. About 50-60% of cats with ascites have FIP. Shortness of breath may sometimes occur due to the accumulation of fluid in the pleural cavity (11).

The diagnosis of FIP constitutes a challenge for veterinarians. The diagnostic process may involve ultrasound, blood chemistry, protein electrophoresis, rapid diagnostic tests, the Rivalta Test (in the effusive form), as well as molecular and serological techniques. In many cases, even positive test results do not allow differentiation between enteric virus and the FIP-causing virus; therefore attempts are made to develop new diagnostic methods to improve the disease diagnosis (8, 11).

The aim of the study was to determine the usefulness of FCoV Ab (VetExpert) rapid serological tests in the diagnosis of the effusive form of FIP in cats.

Material and methods

The cats included in the study were divided into two groups. The study group consisted of 40 cats (24 mixed breed cats, 8 European Shorthair, 3 British Shorthair, 2 Sphynks, 1 Persian, 1 Siamese, and 1 Maine Coon) aged 4 months to 1.5 years with a strain of FCoV causing FIP (the presence of the M1058L mutation) in the abdominal fluid determined using PCR. The control group consisted of 15 cats (all were mixed breed cats) aged 2-8 years with ascites developed in the course of other diseases (pancreatitis in 2 animals, hepatitis in 4 animals, circulatory failure in 9 cats). No genetic material of FCoV was identified in the abdominal fluid samples collected from any of the cats in this group using the PCR technique.

Abdominal fluid for serological and molecular tests was collected using the following method. The abdominal cavity puncture site was shaved and disinfected with a chlorhexidine solution and isopropyl alcohol. The fluid was collected under ultrasound control, with the cat lying on its side. The needle was inserted at a right angle, 1-2 cm below the

navel and 1 cm above the white line. The collected fluid was placed in an empty tube and sent for further analysis.

Serological examinations. Serological examination was performed using rapid immunochromatographic tests (Vet Expert Rapid Test FCoV Ab) detecting antibodies to FCoV. The manufacturer declares the test sensitivity to be 96%, and test specificity to be 97.9%. A sample of the abdominal fluid, approximately 10 µl in volume, was applied onto the test plate using a capillary tube, followed by three drops of the buffer included in the test kit. The result of the test was read 10 minutes after it had been performed. The presence of two lines in the result window indicated a positive test result (presence of antibodies to FCoV), while a single control line indicated a negative test result (no antibodies). The validity of the applied serological test was assessed by determining the probability of obtaining positive and negative results (positive and negative predictive value) using WinEpi (Working in Epidemiology) software.

Molecular studies. Total RNA from the abdominal fluid was isolated using the Total RNA Mini kit (A&A Biotechnology, Gdańsk, Poland). cDNA obtained in the process of reverse transcription was used in Real-Time PCR.

Real-Time PCR reaction. A fragment of the S gene was amplified using Real-Time PCR. The quantitative analysis of the studied gene expression was performed using 2 µl of the matrix containing 200 ng of cDNA. The real-time polymerase chain reaction was carried out in 20 µl thin-walled tubes using the DyNAmo HS SYBR Green qPCR Kit (Finnzymes, Finland) enabling a highly specific qualitative and quantitative reaction. PCR was performed by using specific primers (sense 5'-CAATATTACAATGGCATA-ATGG-3', antisense 5'-CCCTCGAGTCCCCGCAGAAAC-CATACCTA-3') for the first reaction and specific primers (sense 5'-GGCATAATGGTTTTACCTGGTG-3', antisense 5'-TAATTAAGCCTCGCTGCACTT-3') for the second reaction. PCR cycling conditions were 30 cycles at 94°C for 60 s, at 50°C for 30 s, and at 72°C for 1 min plus a 7-min extension at 72°C at the end of the reaction. Primer pairs were expected to generate a 598-bp product covering nucleotides 23442-24040 for the first PCR run and a 142-bp product covering nucleotides 23451-23593 (which includes deviant position 23531 – [i.e., mutation M1058L]) for the second run for the first PCR (2).

The 20 µl volume of the initial reaction mixture contained: 2 µl of DNA matrix, 7.2 µl of water, 0.4 µl of each primer (final concentration 50 pM), 10 µl of Master Mix containing the hot start version of the modified Tbr polymerase (*Thermus brockianus*), a buffer for Tbr polymerase, dNTP, MgCl₂ and SYBR Green 1 intercalating dye.

Reactions were performed using a Rotor-Gene3000 thermal cycler, Corbett Research (Australia). The Ct value of the Real-Time PCR products generated on the cDNA matrix was determined for each reaction (the number of amplification cycles after which the fluorescence intensity of the resulting product exceeded the background fluorescence). In order to confirm the amplification specificity, the melting point of the PCR products was determined by gradually increasing the temperature of the reaction mixture from 50°C to 95°C while continuously measuring fluorescence.

Results and discussion

In all cases, the presence of the genetic material of the mutant feline coronavirus was demonstrated in the abdominal fluid collected from 40 cats constituting the study group. The following range of the ct value, 20-21 for individual samples, was observed. (Fig. 1). By assessing the melting point of the obtained amplicons, it can be concluded that the conducted reactions were characterized by high specificity, which was confirmed by the similar melting point value of the PCR products, which was 81.0-81.2°C (in the case of enteric coronavirus, this value is lower, at about 83.3-83.5°C) (Fig. 2). The PCR test did not detect any FCoV genetic

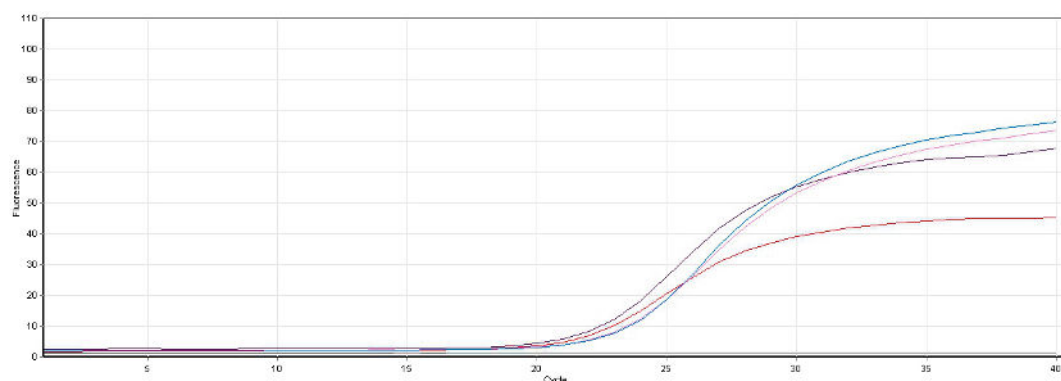


Fig. 1. The ct value of amplicons obtained in own studies

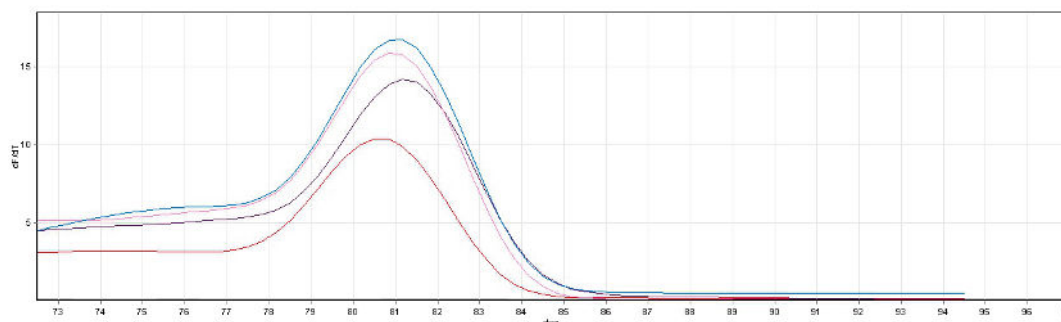


Fig. 2. Melting curves of the of the obtained amplicons

material in any sample of peritoneal fluid collected from the control cats.

Serological examination demonstrated the presence of antibodies to feline coronavirus in 28 out of 40 samples of the fluid collected from animals included in the study group, which constituted 70.0% of the tested samples. The intensity of the colour reaction was high in 19 samples and slightly increased in the other 9 (Fig. 3). No antibodies to coronavirus were identified

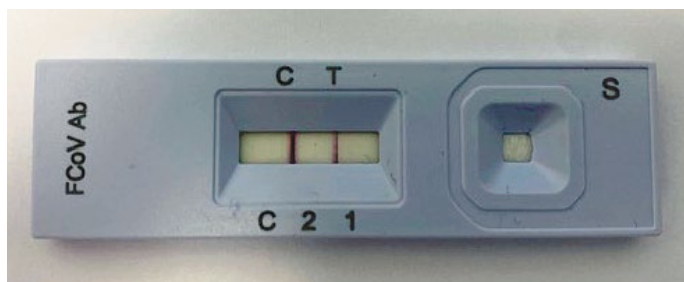


Fig. 3. Positive result of the immunochromatographic test for feline coronavirus infection

in any of the peritoneal fluid samples collected from the cats included in the control group using rapid immunochromatographic tests.

In our own studies, a total of 55 cats were tested (40 cats in the study group and 15 in the control group), 28 of which were infected with feline coronavirus, which allowed for the determination of the apparent prevalence rate, calculated at 50.9%.

As mentioned above, the FIP diagnosis faces many problems, mostly due to the inability to distinguish between enteric coronavirus strains from those causing FIP. Haematological and biochemical tests cannot clearly confirm the form of FIP (12, 13). This disease

is practically always associated with neutrophilia (often with a shift to the left), about half of the cats with FIP suffer from anaemia and/or lymphopenia, and one in three has microcytosis (< MCV). In biochemical tests, nearly 90% of cats with FIP have a hypergammaglobulinemia and/or serum albumin to globulin ratio < 0.8. Of all the above non-specific changes in laboratory test results, an albumin to globulin ratio decrease is relatively often associated with FIP, and rarely with other diseases.

Coronavirus detection using RT-PCR may be helpful if the effusive

form is suspected (4). The detection of this virus in fluid collected from body cavities strongly suggests the presence of FIP. If a non-effusive form is suspected, blood is usually the only test material available. However, a positive RT-PCR result from blood does not necessarily confirm FIP because, as mentioned above, the enteric biotype may also be found in the blood. The diagnostic breakthrough was possible due to the opportunity to differentiate coronavirus biotypes based on the presence of the M1058L or S1060A mutations (2). The detection of one of the two above-described mutations in the peritoneal fluid, the anterior chamber, the cerebrospinal fluid or the biopstat is sometimes regarded as a definitive confirmation of FIP. In our own studies, the presence of FCoV with the M1058L mutation was confirmed in the abdominal fluid from all the cats in the study group. This mutation results from the replacement of adenine at position 23531 of the FCoV S protein gene with thymine or cytosine, which in turn translates into a replacement of methionine to leucine

at position 1058 of the amino acid sequence of the protein. The nucleotide replacement at position 23531 also results in the higher melting point of amplicons from FIPV strains compared to FCoV strains, and therefore they can be differentiated using the real-time HRMSybr Green technique (2).

So far, it has been assumed that serological tests are effective in the diagnosis of FIP to a very limited extent, since antibodies directed against the FIP-causing biotype are indistinguishable from those induced by the enteric biotype. Although cats suffering from FIP often have high titres of anti-coronavirus antibodies, this is not always the case (5-7). As demonstrated by the results of our own studies, the appearance of antibodies in the peritoneal fluid in cats with the wet form of the disease may be considered a confirmation of the disease. In our own studies, the presence of antibodies to the coronavirus was confirmed in the peritoneal fluid collected from 26 out of 40 cats with FIP confirmed using PCR. In cats with ascites, this indicates that, with no access to the PCR technique, a positive result of a rapid test can be considered a confirmation of the disease. This test is simple, quick and easy to perform in an outpatient clinic. The result is obtained in about 10 minutes, and it does not require the use of special reagents. In addition, when examining cats with ascites caused by a disease other than FIP, no false-positive results were observed in these tests. It can therefore be assumed that using this test in cats and verifying the diagnosis with PCR, the predictive value of a positive result (the probability that the cat has FIP) is high, at 98.0%, while the negative predictive value is slightly lower (i.e. the probability that it is not FIP), at 95.8%. The results obtained in our own studies demonstrated that the serological test ensured very high probability, especially in the detection of infected animals, as well as, although with a slightly lower probability, in the exclusion of the presence of FIP virus infection in the samples of fluid collected from the peritoneal cavity. It should be noted, however, that the obtained results relate to our own studies and further research is necessary, involving tests in a larger number of cats, as well as in several different laboratories. This will enable the determination of positive and negative predictive values in other laboratories, as there is a possibility of obtaining different values of the calculated prevalence resulting from a different number of positive results in other laboratories.

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