

## Literature review

# Serological and molecular diagnosis of the feline coronavirus in the americas

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## ABSTRACT

Since its discovery by Holzworth in 1962, the study of the Feline Coronavirus (FCoV) has have a great interest because it can affect wildlife and domestic felines. Currently 2 serotypes are known, type I is unique for felines and type II arose from a double homologous recombination with a canine coronavirus (CCoV); these also can be classified in 2 biotypes, viruses that generate mild enteric diseases (FECVs) and those that cause the feline infectious peritonitis (FIPVs). In the American continent exist different diagnostic methods that together allow the detection of the feline infectious peritonitis (FIP), but the identification of the FCoV only can be done by molecular methods. The countries that have studied this virus the most are those that have the greatest number of tools to carry out diagnostic test, such as United States, Canada and Brazil. In the present work are shown cases reports and the diagnostics methods used to identify the feline coronavirus and/or its biotypes in some countries of the American continent.

**Keywords:** Diagnostic; Feline Coronavirus; FCoV; FIP (*Source: CAB*).

## RESUMEN

Desde su descubrimiento por Holzworth en el año 1962, el estudio del Coronavirus Felino (FCoV) ha sido de gran interés ya que este puede afectar a felinos domésticos y silvestres. Actualmente se conocen 2 serotipos, el tipo I que es único de felinos y el tipo II que nace de una doble recombinación homóloga con un coronavirus canino (CCoV); estos a su vez pueden dividirse en 2 biotipos, los virus que generan enfermedades entéricas leves (FECVs) y los que causan la peritonitis infecciosa felina (FIPVs). En el continente americano existen distintos métodos diagnósticos que permiten en conjunto detectar la peritonitis infecciosa felina (FIP), pero la identificación del FCoV solo se puede hacer por métodos moleculares. Los países que más han estudiado este virus son aquellos que cuentan con una mayor cantidad de herramientas para realizar las pruebas diagnósticas como lo son Estados Unidos, Canadá y Brasil. En el presente trabajo se exhiben los reportes de casos y los métodos diagnósticos usados para identificar el coronavirus felino y/o sus biotipos en algunos países del continente americano.

**Palabras Clave:** Coronavirus felino; FCoV; FIP; diagnóstico (*Fuente: CAB*).

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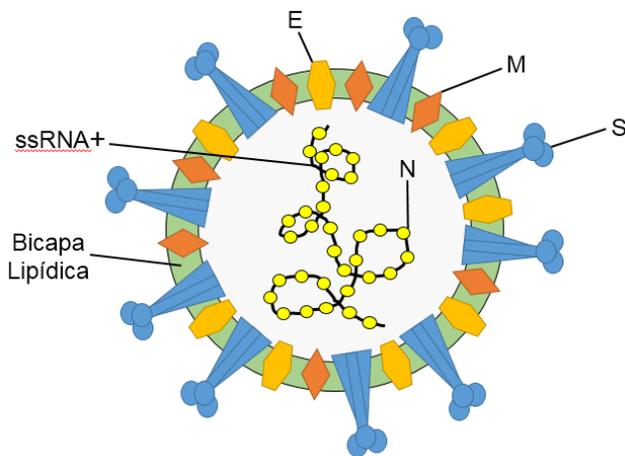
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## INTRODUCTION

Feline coronavirus (FCoV) belongs to the order Nidovirales, family Coronaviridae, subfamily Coronavirinae, genus *Alphacoronavirus* and species *Alphacoronavirus 1*. It is an enveloped virus with a positive sense, single-stranded RNA genome (ssRNA+). Its genome is approximately 30 kilobases (Kb) long with 11 open reading frames (ORFs) that code five accessory proteins (3a, 3b, 3c, 7a and 7b), 16 non-structural proteins that form the viral replication–transcription complex coded in ORFs 1a and 1b; and 4 structural proteins—nucleocapsid (N) protein, spike (S) protein, envelope (E) protein, and matrix (M) protein (Figure 1) (1). The S protein is a glycoprotein in the FCoV envelope. It mediates the virus entry into the host cell by binding to receptors, such as aminopeptidase N (APN) receptor on the cell membrane or C-type lectin receptors on dendritic cells (e.g., DC-SIGN); these are used by the type II FCoV. The M and E proteins are important glycoproteins involved in membrane fusion in the endosome, maturation and assembly of the viral particle, and virus exit. The N protein and RNA form a flexible, helical nucleocapsid (2).



**Figure 1.** Feline coronavirus structure with its associated proteins.

FCoV infections have been described both in wild and domestic felines. The first approach to describing one of the diseases caused by this virus [feline infectious peritonitis (FIP)] was conducted by Holzworth in 1962. In 1968, its viral etiology was confirmed by observing viral particles in the tissue of infected animals. In 1970, Wrad determined that this viral agent was a coronavirus owing to its morphological

similarities to the viral particles of the agents of the family Coronaviridae (3).

FCoV is horizontally transmitted. The feline enteric coronavirus (FECV) biotype has a prevalence of 90% in animals living in environments where there are >2 seropositive felines. Infected animals continuously or intermittently shed the virus in feces for long durations and are typically asymptomatic. Thus, they play a key role in the epidemiology of the virus (4).

FCoVs are classified based on the serotype or biotype. The first classification is based on their serological properties, i.e., the ability to neutralize the virus using specific antibodies against the S protein and the analysis of the S protein gene sequence. The biotype classification is based on pathogenicity, which is defined according to the type of disease cause by them (5).

There are two serotypes. Type I is unique to felines, has an S protein entirely derived from FCoV, and is difficult to propagate in cell culture. Reportedly, in Europe and America, 80%–95% of FCoV infections are caused by type I virus. Type II virus emerged from a double homologous recombination between type I and canine coronavirus (CCoV). This double homologous recombination led to changes in the amino acid sequence of the S protein. The sequence identity between the S1 domain in the S protein from type II and type I serotypes is only 30% (6).

Both serotypes can be further subdivided into two biotypes, FECVs and viruses causing feline infectious peritonitis (FIPVs). FECVs mainly affect enterocytes and cause clinical signs of mild enteritis. On mutation within the FECV genome, they convert to the FIPV biotype. These mutations change their cell tropism, allowing the infection of monocytes and/or macrophages and the spread of the virus (7), subsequently causing FIP (8).

The percentage of occurrence of these internal mutations in FECVs is believed to range between 1% and 5%. Coronaviruses, which are RNA viruses, have an estimated mutation rate of  $4 \times 10^{-4}$  nucleotides/site/year. It has been suggested that mutations resulting in the viral biotype switch occur within the 3c and 7b genes. Similarly, changes in the S protein domains have been assessed because of their role in virus–receptor binding (S1 domain) and viral–cell membrane fusion (S2 domain) during the viral replication cycle. Mutations in these subunits

may lead to modifications in the proteolytic cleavage that would promote alterations of viral cell tropism and development of FIP (9).

The diagnosis of FCoV infection can be performed using serological tests for the detection of antibodies (IgG) with a sensitivity of 95% and a specificity of 83% or using molecular tests that detect the presence of the viral genome. On the other hand, FIP is diagnosed using a combination of methodologies such as animal's clinical record analysis and clinical sign assessment, laboratory tests, effusion tests (if they occur), and diagnostic imaging. Finally, the diagnosis can be confirmed by molecular tests and histopathological analysis (10).

## EPIDEMIOLOGY

The FCoV route of infection is typically oronasal because the virus is shed in the feces and animals get infected by direct contact with these feces or with contaminated fomites. However, studies have shown that FIPVs can be transmitted iatrogenically or under experimental conditions by inoculating fluids from an animal with effusive FIP into a healthy one.

During the early stages of infection, respiratory symptoms may appear mainly in the upper respiratory tract, although in effusive FIP, pleural effusions may cause tachypnea, respiratory distress, and rales (11).

A week after infection, the animal begins shedding the virus, which can continue for several weeks, months, or even throughout its entire life. Approximately 12% of the animals infected with FCoV develop FIP. This disease occurs in felines of any age and breed. FCoV infection is extremely common in highly populated environments where healthy animals often interact with infected animals (12). In such environments, stress-causing factors, such as living with other animals, overcrowding, and competition for food, can contribute to the development of FIP. Other factors that may contribute to its development are those associated with the virus, such as mutations in the S protein, or with the host, such as age, immune response to viral presence, and host cells' ability to maintain the FCoV replication (13).

## VIRUS REPLICATION

FCoV attachment to the cell is mediated by the S protein, which binds to the APN or DC-SIGN cell membrane receptors. These receptors are used by

the type II FCoV. The receptor used by the type I FCoV remains unknown. The virus enters the host cell via endocytosis. After its internalization, the fusion of the viral envelope and the endosomal membrane occurs. Two processes are required to complete this fusion: the proteolytic activation (cleavage) of the S2 domain in the S protein by proteases, such as furins or cathepsins, and a change in the endosomal pH. The release of the viral genome into the cytoplasm initiates the replication process. For this purpose, the transcriptase–replicase complex is assembled. This complex is formed by 16 non-structural proteins (NSPs) that result from the cleavage of the polyproteins pp1a and pp1ab. All genes of the machinery required for this process, including the RNA-dependent RNA polymerase, are coded by ORFs 1a and 1b. The FCoV genome, which is ssRNA+, is copied into a ssRNA– intermediate molecule for the transcription of the structural and accessory genes into subgenomic RNAs (sgRNAs). The glycosylation process of the structural proteins and assembly of the new viral particles occur in the endoplasmic reticulum and in endoplasmic reticulum–Golgi intermediate compartment with the passage of proteins. On completion of this process, the assembled viral particles are transported via secretory vesicles to the cell membrane and exit the cell by membrane fusion (3).

## FELINE CORONAVIRUS INFECTIONS

FECVs cause enteric diseases that are typically asymptomatic or with mild symptomatology. These viruses present intestinal cell tropism and can be detected in fecal matter and blood within few days after infection. Experimental infection studies have shown that the lower part of the intestinal tract is the main site for FECV replication. In FECVs, the route of transmission favors a higher infection in young animals, particularly newborns, which are likely to become infected from the feces of the mother (6).

FIPVs cause FIP, a disease that can be classified into three forms: effusive (wet), non-effusive (dry), and mixed. The general clinical signs observed in FIP are lethargy, weight loss, pyrexia non-responsive to treatments, and jaundice. In the effusive form, an accumulation of a protein-rich fluid in the abdominal and/or thoracic cavities can be observed. This leads to abdominal distension, dyspnea, and tachypnea as well as the presence of a serous layer on the large intestine. Conversely, the non-effusive form lacks these effusions and is primarily associated to the development of neurological, ocular, and dermatological symptoms. Finally, in the third

form, a combination of symptoms of the other two abovementioned forms is observed (13).

Monocytes and macrophages play an important role because FIPVs replicate in these cells and trigger their activation. Circulating activated monocytes express cytokines and cell adhesion molecules that facilitate the interaction with endothelial cells producing endothelial barrier dysfunction, increased vascular permeability, and fluid extravasation, thereby leading to effusions into body cavities (6).

## **MOLECULAR AND HISTOPATHOLOGICAL DIAGNOSIS OF FIP**

FCoV can be detected in tissues, ascitic fluid, serum, and fecal matter. These samples should be carefully handled and frozen to prevent RNA degradation. Diagnosis can be performed using serological and molecular tests (10).

In indirect serological tests, antibodies against the virus are quantified using indirect immunofluorescence tests and ELISA. These tests may present crossreactivity to other alphacoronaviruses. Immunochromatography can be a more appropriate diagnostic method to obtain results similar to the previously mentioned tests in a faster and simpler way (14).

For the molecular detection of FCoV, an RT-PCR is performed using specific primers for the most conserved regions of the viral genome such as RNA polymerase, 7b gene, 3'-UTR, and M and S protein-coding genes. The sensitivity and specificity of this test improves using real time PCR (qRT-PCR). To differentiate the viral serotypes, different sets of primers specific for the S protein gene are used (10).

Similarly, the different viral biotypes can be identified using this technique with a set of primers targeted to the 3c gene. Reportedly, this gene product is truncated in FIPVs; therefore, it could be used as a virulence genetic marker (15).

On the detection of FCoV RNA, it is possible to sequence sections of the genome using techniques such as pyrosequencing and Sanger sequencing. Sequencing the 3c and S protein-coding genes is useful to check for specific mutations in the FIPVs. FCoV can be detected in tissue samples using immunohistochemistry (IHC) or immunofluorescence (IFA). These techniques use fluorochrome-conjugated

antibodies that bind to infected cells and emit fluorescence owing to enzymatic reactions (13).

## **CLINICAL DIAGNOSIS OF FIP**

The age, clinical signs, and physical exploration of the animal should be considered for the diagnosis of FIP. Typically, cats aged between 4 months and 3 years, having jaundice and ascites with abdominal distension, and exhibiting neurological or dermatological signs are suspected of experiencing this disease. Nonregenerative, normochromic, and normocytic anemia; thrombocytopenia; neutrophilic leukocytosis with lymphopenia; increased total serum proteins; and decreased albumin/globulin (A:G) ratio may be found by blood cell count in hematological tests (10).

A blood chemistry panel to determine bilirubin levels is performed on the animal serum or plasma and shows hyperbilirubinemia (mainly in effusive FIP), caused by both hemolysis as well as difficult-to-remove hemoglobin residues. Further, acute phase proteins can be measured, particularly the alpha-1-acid glycoprotein (AGP) that is helpful for the diagnosis of FIP (16).

In effusive FIP, the fluid in the abdominal and/or thoracic cavities can be analyzed. This fluid is collected by puncture and aspiration guided by ultrasound and macro- and microscopically examined. In the macroscopic examination, the consistency and color of the fluid is observed. The color varies depending on the type of pigment—yellow for bilirubin and green for biliverdin. In addition, the degree of turbidity (ranging from clear to cloudy) and protein content are measured. In the microscopic analysis, cell presence is observed and the fluid is classified as an inflammatory exudate if macrophages, non-toxic neutrophils, and lymphocytes are detected (17).

The Rivalta test is used to differentiate effusions due to FIP from other diseases. If a drop of the effusion retains its shape when added to an acetic acid solution, the result is positive; if the drop is diluted, the result is negative. The fluid can be used for serological tests for antibody or antigen detection as well as molecular tests (18).

ELISA has been the most popular test for the detection of antibodies. Healthy cats present antibody titers  $<1/100$ , and infected cats exhibit titers  $\geq 1/400$  (16).

## CORONAVIRUS IN THE AMERICAS: DETECTION USING SEROLOGICAL AND MOLECULAR TESTS

Countries that have reported the detection of FCoV and/or any of the diseases it causes in wild and domestic animals and diagnostic methods used are mentioned below.

**Argentina:** FIP cases have been described. For diagnosis, the animal was first examined for clinical signs. Subsequently, several tests were performed such as hemogram, blood chemistry panel, urine tests, diagnostic imaging, and effusion tests (if they occurred); if the animal died and a necropsy could be performed, the disease was confirmed by histopathology. In 2012, a study was conducted to determine antibodies against FCoV in Geoffroy cats and domestic cats from Parque Nacional Lihué Calel, Parque Nacional Campos del Tuyú, and nearby areas, using the KELA method (kinetics based on ELISA). Only one Geoffroy cat was positive with an antibody titer of 1/12 and all domestic cats were negative (19).

**Bolivia:** In 2004, domestic felines and canines as well as non-domestic carnivores from the areas nearby or within protected areas were studied to determine their antibody titers for different pathogens, including FCoV. The KELA method was used to detect antibodies against FCoV, although no antibodies were detected in any of the animals, cited by Alexander et al (20). In 2018, Napolitano et al aimed to detect antibodies against FCoV in an Andean cat using the FCoV ImmunoComb Kit (IgG) and obtained negative results (21).

**Brazil:** The first reports on FIP prevalence were published in 2003. They observed histological macroscopic and microscopic wounds related to the disease in tissues collected from necropsies of 638 felines between 1970 and 2001. Of these animals, 2.03% (13/638) were diagnosed with FIP, 61.53% of which presented the effusive form and 38.47% the non-effusive form. It was proposed that the collected data (breed, place of origin, and age) could represent potential factors to account for a higher predisposition of the animals to develop the disease, cited by de Oliveira et al (22).

Other studies have included the serological detection of FCoV in wild captive and free cats using IFA test. In 2003, the detection of FeLV and FIPV was described in blood samples. They observed that 12 out of the 16 samples studied were positive for FIPV, concluding that this virus was widely distributed in wild cats in Brazil, cited by Furtado et al (23). In another study, the presence of antibodies against several pathogens, including FCoV, in serum or plasma was investigated. The results were positive for 65% of the captive animals, and it was concluded that this can be attributed to their constant contact with domestic felines (24).

Molecular tests have also been used to detect FCoV in wild cats. Blood samples collected from 1999 to 2011 and using RT-PCR obtained negative results for FCoV (25). Anew, using RT-PCR for FCoV detection in 29 fecal matter samples from domestic felines was observed that only one animal tested positive and two other animals presented a co-infection of FCoV and another pathogen (26). By the end of 2018, the FCoV genome was isolated from fecal samples from domestic cats and fully sequenced using Illumina sequencing. This genome sequence was deposited in GenBank under the accession number MH817484 (27).

**Canada:** In 1969, the first case of FIP was reported. It was on a domestic feline that presented diarrhea, high temperature that gradually worsened, anemia, distended abdomen with fluid (ascites), and poor appetite. Ultimately, the animal died. In the necropsy, macroscopic examination revealed that the fluid in the thoracic and abdominal cavities was yellow, transparent, and viscous. Moreover, the microscopic examination showed that it contained neutrophils and fibrin. Histologically, the detected lesions were related to fibrinonecrotic peritonitis, pleuritis, and varying stages of inflammation in other organs, cited by Lauzi et al (28).

In 1982, was observed viral particles of FCoV in the fecal matter from a domestic feline. Electron microscopy was used for diagnosis; the viral particles visualized were pleomorphic in shape, enveloped, and ranged from 70 to 150 nm in size. Using counterimmunoelectroosmophoresis (CIE), the detection of the antigen was confirmed by the formation of a precipitation line (29).

A study conducted from 1993 to 2001 sought antibodies against several pathogens, including FCoV, in 215 Canada lynxes. Using IFA tests, the exposure of these animals to FECV and FIPV biotypes was confirmed. Some animals showed high antibody titers, suggesting that they had recently been exposed to these biotypes. The prevalence of the virus showed no variation according to the age, sex, or regional distribution of the animal, cited by Licht et al (30).

In 2013, Bauer et al (31) reported a case of FIP in an animal presenting with multiple skin lesions, bilateral panuveitis, anorexia, and lethargy. In addition to the routine tests (hemogram, blood chemistry panel, and serum protein electrophoresis), ELISA was performed to measure antibody titers against different feline viral agents. Titers of 1/51200 for FIPV were obtained. Biopsies were performed from skin lesions and FCoV was detected in dermal macrophages using IHC and confirmed by histopathological diagnosis (31).

In 2020, McKay et al (32) sought to determine whether mutations in the S protein that are considered to be FIPV-specific (M1030L and/or S1032A) can be found in domestic felines diagnosed with FIP at postmortem examination. To this end, 185 samples from fecal matter, 63 from tissue, and 2 from ascitic fluid were collected and RT-PCR tests were performed. Positive samples were sequenced by Sanger sequencing for further phylogenetic analysis. Of the 185 samples, 46% (86/185) were positive for type I FECV and 26% (49/185) for FIPV, 8 of which contained the M1030L mutation and 1 contained the S1032A mutation (32).

**Chile:** In 1985, a case of suspected FIP was reported. Laboratory tests and an abdominal X-ray were performed, and fluid was detected in the cavity. After a week, an exploratory laparotomy showed that this fluid was rather cloudy, foamy, and viscous. A sample was obtained, laboratory analysis was conducted, and FIP was confirmed (33).

**Colombia:** Two cases of effusive FIP have been reported and confirmed by necropsy and effusion tests. In the first case, during the necropsy, macroscopic examination revealed a

fibrinous exudate on the stomach and peritoneal surfaces and microscopic examination revealed an enteritis with shortening and destruction of the small intestine villi (34). In the second case, yellow and viscous fluid was adhered to the lung pleura and peritoneum. Additionally, a histopathological analysis on the tissue samples showed renal perivasculitis, acute pneumonia, and fibrin deposits in the spleen. These findings are common in necropsies performed on cats with effusive FIP (35).

In 2013, Ramirez et al (36) determined FCoV seroprevalence in stray, household, and shelter cat populations using ImmunoComb® Lab ELISA Kit, Biogal. Of 150 samples, 93 were positive, i.e., a seroprevalence of 62% was observed (36). The same test was used in wild cats by Fletcher et al in 2016; they observed a seroprevalence of 10.71% (37).

In 2017, Delgado et al (38) studied FCoV prevalence in felines from different shelters; 96 samples were collected, and ELISA was used; a prevalence of 84.64% was observed. Further confirmation was obtained using RT-PCR targeted to the Nsp14 gene. Subsequently, samples that were positive for both tests were sequenced by Sanger sequencing, and type I FCoV serotype was identified by phylogenetic analysis (38).

From 2014 to 2018, Santana et al (39) collected effusion samples from 5 felines and fecal matter samples from 44 canines for coronavirus testing. The samples were analyzed using RT-PCR targeted to the Nsp12 gene followed by partial amplification of the M, N, S and 3b protein-coding genes. Only one cat was positive for FCoV, and according to the phylogenetic analysis based on the M protein-coding gene, it was classified as type II FCoV, i.e., it was grouped with a reference sequence of type II FCoV in the maximum likelihood tree (39).

**Ecuador:** A study to diagnose FCoV and other pathogens was conducted in Isla Isabela, Galapagos. Samples were collected from 95 dogs and 52 cats, and antibodies against FCoV were analyzed using ELISA. All the studied felines were negative for the virus, cited by Teiseira et al (40).

**United States:** In 1968, Zook et al (41) visualized, for the first time, viral particles in lesions caused by FIP using electron microscopy in tissues from experimentally infected domestic felines. These animals were periodically observed and examined until the time of their death. Eventually, a necropsy was performed and samples were collected from different tissues. Overall, 17 animals developed the disease, and viral particles were observed in mesenteric mesothelial cells using electron microscopy (41).

Different techniques, such as IFA, ELISA, and viral neutralization, were used to evaluate the immune response against FCoV, although ELISA exhibited some shortcomings. Accordingly, the KELA test was developed to detect antibodies for different pathogens. In 1982, a computational adaptation of this test to detect antibodies for FCoV was conducted and determined that it decreased the ELISA requirement of serial dilution of serums. This consequently minimized the errors from physical manipulation of the sample and provided faster and more accurate results, cited by Domínguez et al (42).

In 1990, was assessed the FCoV prevalence in captive cheetahs using IFA tests. Animals that tested positive showed FIPV antibody titers  $\geq 1/25$  and were subsequently confirmed using western blot. Further, FCoV prevalence in fecal matter was found to be 31%, cited by Kim et al (43). IFA and KELA tests were used in 1993 to detect antibodies against FECV and FIPV in Florida panthers. Positive results were obtained for 4 out of 21 animals using IFA test, whereas all animals showed negative results using KELA. In addition, FCoV prevalence in panthers was 19%, cited by Foley et al (44).

In 2001, a study in wild felines (captive cheetahs) to detect FCoV in blood, fecal matter, and effusion fluid samples obtained from 33 cheetahs was done. They used RT-PCR targeted to the accessory proteins from ORF 7ab, WSU1143 strain (American BioResearch, Sevierville, Tennessee 37864, USA) as positive control and RNase free water as negative control. Ten samples were positive. An IFA test was also performed, which demonstrated that 13 samples were positive for serotype I and 2 for serotype II, cited by Gaffney et al (45).

Similarly, in 2014, a qRT-PCR targeting the 7b gene was performed and 88% of the 68 animals tested were positive for FCoV (46).

Additionally, PCR tests can be complemented by sequencing of the products obtained to develop complete or partial genomes of the virus and subsequently establish the phylogenetic relationships (47).

**Guatemala:** In 2001, blood samples were collected from 30 domestic felines and 2 captive margays in the Petén region to determine antibodies for FCoV and other feline pathogens. Antibody titers were detected using IFA tests that yielded 27% seropositive for domestic felines with titers of 1/40. Similarly, both sampled margays were positive for FCoV with titers of 1/40 and  $< 1/80$  (48).

**Peru:** Until 1997, no information about diseases caused by FCoV or cases of FIP were reported in this country; thus, a study was conducted with the aim of proving the existence of FCoV infections in Lima. Antibodies detection by IFA tests showed that 27.8% of the animals were positive for type I. In addition, among the animals positive for type I, 11.1% were also positive for type II (49). In 2018, FIP diagnosis was performed by correlation of clinical signs and laboratory tests and confirmed by necropsy and histopathology in two cases of FIP reported by a veterinary clinic. The necropsy showed that both animals had ascites and fibrin masses adhered to serosa of the liver, spleen, pericardium, intestine, and mesentery. Histopathology showed an inflammatory exudate composed of fibrin threads; vasculitis, and protein fluid effusion; necrotic areas in the liver; and lymphoid follicles with moderate depletion in the spleen. These findings confirmed that both cases were effusive FIP (50).

**Venezuela:** In 2005, blood and fluid samples from the thoracic and abdominal cavities of three domestic felines with FIP symptoms were tested. Subsequently, a necropsy was performed and tissue samples were obtained for histopathological analysis. The fluid was whitish or amber, cloudy, and aqueous and contained fibrin and high protein content. Macroscopically, adhesions were observed

between the parietal and visceral pleura and microscopically, infiltration of inflammatory cells was observed in the bronchi, visceral pleura, and intestinal serosa. These alterations suggested the diagnosis of effusive FIP (51).

## CONCLUSIONS

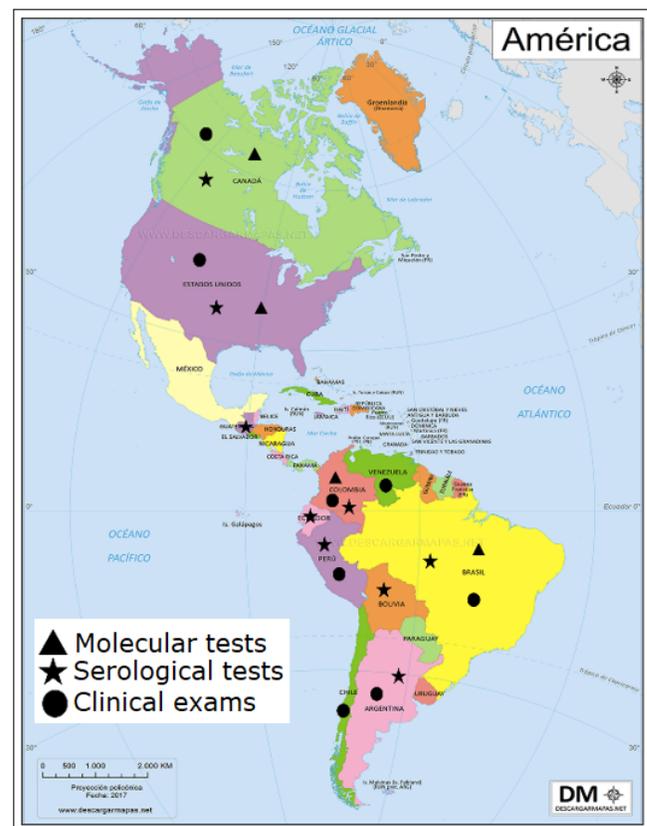
The prevalence of FCoV ranges from 20% to 90% in wild and domestic feline populations and causes diseases, such as FIP or intermittent enteritis, throughout the animal's life. This virus has more frequently been isolated from young animals living in overpopulated environments owing to the oronasal transmission of the virus. There are two serotypes of the virus—type I and type II. The first one is found only in felines, whereas the second one emerges from a double homologous recombination between type I and CCoV. These two serotypes can be further subdivided into two biotypes: FECVs that cause mild enteritis and FIPVs that cause FIP. Most research is focused on the latter, for which daily routine clinical analysis, such as hemograms, biochemical tests, effusion tests, serological tests to determine titers of antibodies against the virus, and molecular tests, are conducted. Molecular tests are the most effective ones for detecting the viral genome. Moreover, histopathological alterations can be found in the animal necropsy that suggest the diagnosis of this disease.

As previously described, the prevalence of FCoV or FIP has been investigated in less than half of the countries in the American continent. More developed countries employ a wide variety of tests to detect FCoV, thereby resulting in further research being conducted (Figure 2). Conversely, developing countries have performed less research on this topic. This may be owing to the lack of economic interest in pets because they are typically considered as companion animals.

Countries that are not mentioned in this article should not be disregarded. They may use one or more of the methods described above; however, they are not mentioned herein owing to the difficulty to obtain information about them or the lack of publications on this issue. Similarly, some of the countries mentioned in the

present study provide limited information about the FCoV screening. Therefore, information on the prevalence of FCoV in the Americas is scarce, which hampers the possibility of finding a diagnostic test for the exact and accurate detection of this virus.

Notably, several countries do not use molecular tests as the primary method to detect FCoV and other feline diseases have symptoms similar to those observed in diseases caused by both FCoV biotypes. Therefore, considering the higher accuracy required for the detection of this pathogen, it is necessary to implement molecular tests as routine diagnostic method. In addition, serological tests often produce results that are not 100% confirmatory, leading to erroneous diagnosis of the infection.



**Figure 2.** Representation of the American continent and the diagnostic methods used for detection of FCoV infection

## Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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