**Diagnosis of feline infectious peritonitis by immunohistochemistry and histopathology methods: A case report based on diagnostic approach**

**Abstract**

In this study the case of feline infectious peritonitis (FIP) has been identified as histopathological and immunohistochemical (IHC) in a 6-month-old male domestic shorthair cat. In necropsy, the abdominal cavity contained a significant volume of yellow liquid and granular fibrinous exudate was seen on the liver, serosa of intestines, and peritoneum. Microscopically, inflammatory cell infiltration and fibrin exudation, consisting mainly of macrophage, lymphocyte and plasma cells, were determined around small and medium-sized vessels in the tunica serosa layer of the intestines. Many granulomatous foci of various sizes, with or without necrosis, were found in the liver, spread to the parenchyma. In methyl green pyronin staining, plasma cells were found to be the majority of the inflammatory cells present in lesions. In IHC staining with specific antibodies against the agent, immunopositivity was obtained in granulomatous lesions in the serosa layer of the intestines and less frequently in the cytoplasm of mononuclear cells in the lamina propria. Immunoreactivity was detected in the cytoplasm of macrophages in the liver, around both pyrogranulomas and granulomas. It was concluded that this case of FIP, when all findings are evaluated together, may have started as a dry form, and turned into a wet form in the terminal period.

**Key words:** Coronavirus**,** peritonitis, dry FIP, pyogranuloma, feline

**Kedi enfeksiyöz peritonitinin immünohistokimyasal ve histopatolojik yöntemlerle teşhisi: Diagnostik yaklaşıma dayalı bir olgu sunumu**

**Öz**

Bu çalışmada, 6 aylık, tekir bir erkek kedide histopatolojik ve immünohistokimyasal (IHC) yöntemlerle kedi enfeksiyöz peritonit (FIP) vakası, tanımlanmıştır. Yapılan nekropside, karın boşluğunda önemli miktarda sarı renkli bir sıvı birikimi ile karaciğer, bağırsak serozası ve peritonda granüler tarzda fibrinöz bir eksüdat görüldü. Mikroskobik olarak bağırsakların tunika serosa tabakasındaki küçük ve orta çaplı damarların etrafında yoğun olarak makrofaj, lenfosit ve plazma hücrelerinden oluşan yangısel hücre infiltrasyonu ve fibrin eksüdasyonu belirlendi. Karaciğerde ise paranşime yayılmış, çeşitli boyutlarda nekrozlu veya nekrozsuz birçok granülomatöz odak bulundu. Methyl green pyronin boyamasında, plazma hücrelerinin, lezyonlarda bulunan inflamatuar hücrelerin çoğunluğunu oluşturduğu tespit edildi. Etkene karşı spesifik antikorlarla yapılan IHC boyamasında, bağırsakların serosa tabakasındaki granülomatöz lezyonlarda ve daha az sıklıkla lamina propriadaki mononükleer hücrelerin sitoplazmasında immünopozitivite elde edildi. Karaciğerde ise hem granülom hem de piyogranülomların etrafındaki makrofajların sitoplazmasında immünoreaktivite belirlendi. Tüm bulgular birlikte değerlendirildiğinde bu FIP olgusunun kuru form olarak başlayıp terminal dönemde ıslak forma dönüştüğü sonucuna varıldı.

**Anahtar kelimeler:** Koronavirüs, peritonit, kuru FİP, piyogranülom, kedi

**Introduction**

Feline infectious peritonitis (FIP), one of the leading fatal diseases of cats and created by feline coronaviruses (FCoV), is seen especially in cats with a young and immune system that is not strong enough or suppressed. This disease is a lethal, systemic and infectious viral disease whose pathogenesis has not been fully revealed (Kipar and Meli 2014). It is assumed that the disease develops by mutation of the cat enteric coronaviruses (FECV) in the host or by receiving externally derived FIP virus strains (FIPV). Feline infectious peritonitis virus (FIPV) has been reported to reproduce by infecting monocyte / macrophages and vascular endothelial cells, and they form a systemic infection through the blood (Kipar et al 2005, Pedersen 2009). This results in clinically effusive FIP or non-effusive FIP, depending on the host's immune response. It is also observed in mix form (Kipar and Meli 2014). It has been reported that “dry (parenchymatous / non-effusive) FIP which is the chronic form of the disease, occurs when the immune response is insufficient, but “wet (nonparenchymatous / effusive) FIP” develops if the immune response never occurs (Ciftci et al 2018). In naturally occurring FIP cases, the clinical course of the wet form develops rather quickly (2-14 days), while in dry form it develops slowly (several weeks) (Pedersen 2009). While peritonitis is formed in both forms, the wet form is characterized by protein-rich effusions in the abdominal cavity and / or chest cavity, and the dry form is characterized by granulomatous inflammation in various organs (Kipar et al 2005, Ciftci et al 2018). Another important microscopic lesion of the disease is vasculitis or perivasculitis, which is observed in small and medium-sized venules in the affected tissues and organs (Uzal et al 2016). The *ante-mortem* diagnosis of FIP, especially its dry form, is very difficult. Histopathological examination and immunohistochemical (IHC) representation of FCoV antigens, which are usually localized around the lesions, are accepted as the gold standard for the definitive diagnosis (Hartmann et al 2003, Pedersen 2009, Tasker 2018).

In clinical practice, FIP diagnosis remains an important problem. Preliminary diagnosis is being attempted with a complete anamnesis, clinical review, and findings of certain diagnostic tests such as serum biochemical parameters, antibody titer measurement, Real Time-PCR or ELISA. For this, it is necessary to make a definitive diagnosis for FIP. In fact, histopathology, and immunohistochemistry-based diagnosis, which is considered the gold standard for the diagnosis of FIP, are very limited in veterinary clinics. Therefore, we think that these methods should become widespread for the definitive diagnosis of FIP which is quite common and fatal in cats. In this case report, we review the literature and describe clinical, *post-mortem*, and microscopic findings a rare case of mixed FIP that includes both the dry form and the wet form.

**Case presentation**

In this case report a FIP case was presented in a 6-month-old male domestic shorthair cat. Owing to anorexia, fever, vomiting and diarrhoea, the cat has been given antibiotics, anti-inflammatory and supportive therapy for a week. After the treatment, these symptoms decreased for a short period of time, but later the enlargement of the abdomen took shape and the cat died without showing any another clinical symptoms. Later, this cat was brought to the XXX for necropsy. During systemic necropsy, fixation of the samples taken from the lung, liver, brain, eyes, heart, spleen, kidney and intestines and lymph nodes for histopathological and immunohistochemical examination was provided with 10% buffered formaldehyde. Also, tissue samples were taken for microbiological examinations during the necropsy. After routine tissue processing steps taken from paraffin-blocked tissues were stained with haematoxylin-eosin (HE) and methyl green pyronin (MGP) for identification of plasma cells in tissue sections. Also, tissue samples were taken for microbiological examinations during the necropsy.

In addition, immunohistochemical (IHC) staining was performed using the FIPV-specific primary antibody (FIPV3-70, sc-65653, monoclonal, 1: 100, Santa Cruz Biotechnology, Texas, USA) to selected tissue sections. Sections for this staining were placed on the fully automated immunohistochemistry staining device (Bond max, Leica Biosystems, Buffalo Grove, USA) and the commercial kit (Refine Detection Kit, DS-9800, Leica Biosystems, Newcastle, UK) procedure was performed. All prepared preparations were examined under a light microscope.

In the macroscopic examination, a fluid of approximately one liter of yellow colour with an egg-white consistency containing white clots in the abdominal cavity was determined (Figure 1A-B). The serosa layers of all intestinal section covered with fibrinous exudate was determined to have a matte appearance. In addition, yellow-white coloured nodular structures, which could be approximately 0.5 cm in size from the pinhead size were observed on the intestinal serosa (Figure 1C). Similarly, a granular-looking fibrinous serositis was detected in the peritoneum. Thickening of the edges of the liver, darkening in colour and fibrin plaques were detected on it. A variegated image was encountered due to diffuse grey-white coloured necrotic foci spread in the parenchyma on the cut-section of the liver (Figure 1D). The lungs were found to be voluminous. Slight growth was observed in the kidneys and easily separable fibrin fibers were detected in the capsule. There were no macroscopic findings in the brain, eyes, and other organs.

In histopathological examination, fibrinous perihepatitis consisting of fibrin and locally inflammatory cell aggregates were observed in the capsule of the liver (Figure 2A). Many granulomatous foci of different sizes, with or without necrosis, were found in the parenchyma. It was observed that parenchymatous foci without necrosis consisted of intense macrophage, lymphocyte, plasma cells infiltration (Figure 2B). In necrotic granulomas, it was found that large coagulation necrosis areas with karyorrhectic macrophage and neutrophil granulocytes (Figure 2C). Occasional haemorrhage was detected around these pyogranulomas. Similarly, it was noted that perivascular pyogranulomas were formed around the central vein in the liver (Figure 2C). In the portal area, mononuclear cell infiltration was determined. Furthermore, hydropic degeneration and intrahepatic cholestasis were observed in hepatocytes. In the intestines, there was degeneration and desquamation in the lamina epithelialis, as well as oedema and mononuclear cell infiltration in lamina propria. Dense fibrin exudate and inflammatory cell infiltrations have been observed in tunica serosa, beginning around small and medium-sized venae and it has been determined that these spread to the tunica muscularis. Additionally, nodules formed by macrophage, lymphocyte, plasma cells infiltration in serosa were identified (Figure 3). In MGP staining, some of inflammatory cells in lesions in both liver and intestines were found to be plasma cells (Figure 2D). Thickening of the capsule due to mild fibrin exudation, hydropic degeneration in tubules epithelium and protein-rich fluid in tubules lumens were detected in the kidneys but no granulomatous lesions were observed. Thickening in the interalveolar septum due to mononuclear cell infiltration and oedema in some alveoli were detected.

Immunohistochemical examination revealed viral antigen in macrophages in parenchymatous foci without necrosis in the liver (Figure 4A). On the other hand, positive immunoreactivity was observed in macrophages limiting necrotic pyogranulomas (Figure 4B). Similarly, positive staining was obtained in inflammatory cells in portal areas. No immune reaction was found in the epithelial layer in the intestines. It was determined that the immunoreactivity was limited only to macrophages, lymphocytes and plasma cells in the lesions in the serosa layer of the intestines and to a lesser extent in macrophage in the lamina propria and not in enterocytes. Positive immune staining was not found in the kidney and other tissues. Another noteworthy situation was the strong immune reactions in plasma cells, which are the major inflammatory cells in both the liver and intestines (Figure 4A-D). It was reported that no bacteria were found in the bacterial culture, possibly due to the use of antibiotics.

Feline infectious peritonitis is a very common disease in the cat population, but it is often undiagnosed and its pathogenesis cannot be fully revealed. FIP continues to be one of the most important infections of cats due to the absence of a direct diagnostic test for diagnosis, lack of protection vaccines and uncertainty of the virus and host interaction of the disease (Pedersen 2014).

In our study, findings such as anorexia, vomiting, fever, and diarrhoea, which are clinically observed and evaluated as classic gastroenteritis, are not specific for the diagnosis of FIP. Although not evaluated in this study, it has been reported that parameters such as neutrophilia, lymphopenia, thrombocytopenia, anaemia, hyperbilirubinemia, hyperproteinaemia and hypergammaglobulinemia, and clinical-pathological parameters such as liver enzyme activity, blood urea nitrogen and creatinine are not pathognomonic for FIP (Hartmann et al 2003). With the abdominal enlargement encountered in *post-mortem* examination, the yellow-coloured fluid in the peritoneal cavity, with white clots in the consistency of egg white, is considered as the most prominent macroscopic finding of effusive (wet) FIP (Pedersen 2014, Pedersen 2019). However, it is known that this fluid is found only in half of the cats suffering from FIP (Hartmann et al 2003). It should also be remembered that similar effusions may occur in heart, liver diseases, lymphatic drainage disorders or neoplasms. In other words, determining fluid accumulation in body cavities during *ante-mortem* or *post-mortem* examination is not enough for FIP diagnosis, whereas the absence of these findings is not sufficient to rule out this disease too. It was reported that macroscopically, peritoneal, intestinal and liver serosa were covered with fibrinous exudate and observation of grey-coloured granular structures with varying diameters was observed more in effusive FIP and less in dry FIP (Kipar et al 1998, Pedersen 2009).

Histopathologically, lesions ranging from multifocal inflammatory cell infiltrations to large granulomas without necrosis or necrosis, and sometimes even lesions that merge with each other, were detected in the liver. Furthermore, large necrotic granulomas were found beginning around the central vein. Significant lymphoplasmacytic accumulation in the portal area attracted attention. In the centre of some granulomas, wide areas of necrosis and karyorrhectic macrophage and few neutrophils were identified, with a thin line around macrophages, lymphocytes, plasma cells, and a small number of neutrophil granulocytes. Other in the parenchymatous granulomas without necrosis, were macrophages in the centre, and dense lymphocytes and plasma cells around them. It has been reported that pyogranulomas formed by neutrophils and other inflammatory cells are more commonly seen in wet FIP. In contrast, antigen-filled macrophages in the centre of typical granulomas with lymphocytes (mostly B-lymphocyte) and plasma cells around them are in dry FIP and there are not necrosis and neutrophil. (Pedersen 2009, Pedersen 2014). Based on these, the presence of both types of granulomas, especially in the liver as described above, was evaluated as a mixed form of FIP.

Immunologically, it has been stated that if the immune response of the body to the viral antigen is mostly humoral, wet FIP develops and dry FIP develops if weak cellular immunity (partial immunity) occurs (Pedersen 2009). It has also been reported that most of the pathology occurring in FIP is due to the inability of cellular immunity to strongly accompany intense humoral immunity (Hartmann 2005, Pedersen 2014, Tasker 2018). In our study, the methyl green pyronine staining revealed that many of the cells surrounding the granulomas are plasma cells. It was noted that these plasma cells were scattered clustered around the veins in the portal area of the liver and intestinal serosa. In addition, histopathological examinations revealed that cells other than plasma cells infiltrated around the lesions and vessels were proportionally less. All of these were accepted as findings showing that humoral immunity is more effective than cellular immunity in inflammation in the case.

Although evaluation of histopathological findings is very important for FIP, immunohistochemical confirmation of the diagnosis is recommended (Kipar et al 1998, Kipar and Meli 2014). The IHC method has been reported to be 100% diagnosed in cats with both FIP forms by detecting FCoV antigen in the cytoplasm of macrophages (Kipar et al 1998, Hartmann et al 2003). In the presented case, positive staining was obtained in macrophages localized both in the centre of non-necrosis granulomas and around the granulomas with necrosis. In addition, sometimes immune positivity was obtained against free antigen in areas with lesions. It has been observed that this staining model and its localization are compatible with previous studies (Kipar et al 1998, Giori et al 2011, Malbon et al 2019). In a study of 488 cats diagnosed as FIP histopathologically, the only way to definitively diagnose FIP was reported to be histopathology and detection of intracellular FCoV antigen by immunofluorescence or immunohistochemistry staining (Hartmann et al 2003). Furthermore, it has been reported that IHC with FCoV antibody is compulsory to confirm or exclude the disease in suspicious cases (Hartmann 2005, Pedersen 2009).

Another important IHC finding in our study was the positive immunoreactivity in plasma cells, which is the main cell type collected in granulomas and perivascular areas. As Kipar et al (1998) reported, the presence of these plasma cells filled with antibodies specific to the corona virus is a result of continuous antibody synthesis against the agent. This is an indication that strong humoral immunity is effective in this infection. The dominantly shaped humoral immunity in this way has also been interpreted to contribute to type III hypersensitivity reactions (Arthus reaction) that are formed against the virus and direct the formation of FIP lesions (Kipar et al 1998, Pedersen 2009, Kipar and Meli 2014). In addition, the absence of any immunoreactivity in the enterocytes in the intestine may indicate that the FECV mutation to FIPV, which shows tropism to macrophage/monocytes, is not within the enterocytes.

In a study, it was determined that real-time (RT) PCR was used for diagnostic purposes (Baydar et al 2014). However, RT-PCR testing has been reported to not be a reliable tool for diagnosing this disease (Can-Şahna et al 2007, Tasker 2018). Because the amplification of too few viral genetic materials that cannot cause disease from samples taken with RT-PCR, which is a very sensitive test, does not mean that the infection exists (Can-Şahna et al 2007). It has also been informed that this test cannot differentiate between FECV and FIPV (Aytug 2008). In another study, the prevalence of coronavirus antibodies in cats was tried to be determined by using ELISA method (Pratelli et al 2009). However, it has been reported that many clinically healthy cats usually have very high FCoV antibody titres, while ~ 10% of cats with FIP are seronegative (Tasker 2018). In other words, the presence of seropositivity in such tests does not confirm that a cat has FIP and does not eliminate the disease because it is negative (Aytug 2008).

In experimental infections, it has been informed that the dry form always follows a short-term effusive form, that is, there may be transitions between the two forms of FIP (Pedersen 2014). In dry form, central nervous system and eye disorders are reported to occur in 60% of cases (Pedersen 2009). Also, the tendency of dry FIP lesions to spread from serosal or pleural surfaces to organ parenchyma is known (Pedersen 2009). Microscopically, it was emphasized that the non-effusive form was characterized by typical granuloma while the effusive form was characterized by pyogranulomas (Hartmann 2005, Pedersen 2014). When all of these are evaluated, the formation of death after abdominal enlargement and fluid exudation approximately one week after the first symptoms, the tendency of the distribution of lesions in the liver to spread to the organ parenchyma, and microscopically, the presence of both pyogranuloma and typical granulomas suggested that the disease may have started as a dry form and turned into a wet form in the terminal period although there are no neural signs or eye disorders.

As a result, it is very important to make a definitive diagnosis of FIP, which is observed quite widely in the lethal and cat population and whose pathogenesis has not been elucidated yet. In the study, it has been revealed that histopathological examination and the combination of IHC provide unique information for the diagnosis of this disease. During the disease, the inability of the cellular immune response to adequately accompany humoral immunity and excessive production of antibodies triggers Arthus-type hypersensitivity, possibly worsening the prognosis of the disease. Also, failure to fully function the virus-infected macrophage/monocytes has been thought to contribute to this situation. It was thought that histopathological, immunocytochemistry and immunohistochemistry examination, which will be performed not only on *post-mortem* tissues, but also on laparoscopic or ultrasound-guided biopsies especially in asymptomatic cases, can contribute significantly to the confirmation of the disease and these methods should become widespread.

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| **Figure 1 insert here** |
| **Figure 1.** Macroscopic examination findings. A: Severe abdominal enlargement, B: Fluid containing fibrin clots in the abdominal cavity, C: Yellow-white coloured nodular structures on the intestinal serosa, D: Diffuse grey-white coloured necrotic foci spread in the parenchyma. |

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| **Figure 2 insert here** |
| **Figure 2.** Histopathological examination findings. A: Fibrinous perihepatitis and necrotic foci spreading into the parenchyma of the liver, HE, 10X, B: Granulomatous foci without necrosis in the liver, HE, 20X, C: Perivascular pyogranuloma in the liver, HE, 10X, D: Many plasma cells with red cytoplasm in the serosa of intestine, MGP, 20X.  |