Acta Veterinaria-Beograd 2023, 73 (3), 432-438
UDK: 636.8.09:616.98-097
DOI: 10.2478/acve-2023-0032

Short communication

IMMUNOHISTOCHEMICAL INVESTIGATION OF FIPV3-70 ANTIGEN EXPRESSION IN THE ILEUM OF CATS WITH EFFUSIVE FELINE INFECTIVE PERITONITIS

Todor NOVAKOV[1]*, Ivica GJUROVSKI[1], Spiro BOZINOSKI[1], Aleksandar JANEVSKI[1], Elena ATANASKOVA PETROV[1], Slavica KOSTADINOVA KUNOVSKA[2], Trpe RISTOSKI[1]

[1]Faculty of Veterinary Medicine, "Ss Cyril and Methodius" University in Skopje, Lazar Pop Trajkov 5-7, 1000 Skopje, Republic of Macedonia; [2]Faculty of Medicine, Ss. Cyril and Methodius University, Skopje, 50 Divizija 6, Republic of North Macedonia

(Received 16 March, Accepted 31 August 2023)

One of the most common infectious causes of cat mortality is feline infective peritonitis (FIP), along with panleukopenia and viral upper respiratory tract infections. FIP is more likely to affect cats whose immune system is weak or suppressed. It is thought that the infection of macrophages and monocytes plays a major role in the pathogenic process. In order to set a definitive diagnosis for this infectious disease, a histopathological examination of tissues, and feline coronavirus (FCoV) detection by immunohistochemistry (IHC) is necessary. In this investigation, 15 cats between the ages of 5 and 24 months with clinical suspicion of FIP, underwent post-mortem necropsy, pathohistological and immunohistochemical examination. The results showed that all the cats had abdominal effusion with pyogranulomas throughout the abdominal serosa. Ten out of fifteen cats were FIP positive using immunohistochemical methods. This method also showed the antigen deposition in the macrophages thus confirming their role in the pathogenesis of FIP.

Keywords: feline infectious peritonitis, Coronavirus pan Monoclonal Antibody FIPV3-70, MAC 387, immunohistochemistry, cat, ileum

INTRODUCTION

Feline infectious peritonitis (FIP), along with viral upper respiratory tract infections and panleukopenia, is one of the most prevalent infectious causes of cat death. Cats with an insufficient or suppressed immune system are more likely to suffer from FIP [1]. This infectious disease is systemic and usually has a lethal outcome, though its' pathogenesis has not been fully elucidated [2]. As a result of a minor mutation, the FIP virus (FIPV) acquired the ability to pass through the epithelium and replicate

*Corresponding author: e-mail: todornovakov@gmail.com

in the lymphocytes from the monocytic-macrophagic lineage. FIPV spreads through the body to target organs from the monocytes [3]. It is believed that the pathogenic process is mostly dependent on the infection of macrophages and monocytes [4]. A diagnostic suspicion of FIP may be based on history, clinical signs, and laboratory analyses, however, to set a definitive diagnosis, a histopathological examination of tissues and feline coronavirus (FCoV) detection by immunohistochemistry (IHC) is necessary [5]. Immunostaining for FCoV antigen should be performed to confirm the diagnosis when histological lesions are not strongly suggestive of FIP [6].

It is still considered the gold standard for the detection of FIP by IHC staining of FCoV antigen in characteristic histopathological tissue lesions. A histopathologically confirmed FIP can be diagnosed with IHC with a sensitivity of 97-100% and a specificity of 100% for excluding FIP in cats with other histopathologically confirmed diseases [2].

Upon gross post mortem examination, fibrinous and granulomatous serositis, protein-rich serous effusions, and/or pyogranulomatous lesions are common signs of FIP [4]. On the basis of their distribution, cellular make-up, and viral antigen expression, four types of lesions were classified: distinctive subserous alterations, granulomas with and without necrosis, focal to necrotizing phlebitis, and focal and perivascular B-cell infiltrations [7].

The aim of this study is to present the immunohistochemical diagnosis of feline corona virus in cats with previous clinical suspicion of FIP.

MATERIAL AND METHODS

Animals

Fifteen cats at the age from 5 to 24 months, which were previously clinically diagnosed as FIP suspected, were examined post mortem. Materials were collected within twenty-four hours after the animals' death. A complete necropsy was performed on all of the cats in order to evaluate the morphological changes of the internal organs as well as to collect tissues for further investigation. Immunohistochemistry for feline coronavirus and macrophages was performed on paraffin sections from all cases. The collection included tissue samples from the intestine which were fixed by immersion in 10% buffered formalin solution and processed for routine histopathological evaluation (H&E stained), as well as immunohistochemical investigation.

Histopathology and immunohistochemistry

The tissue samples from the small and large intestine collected at necropsy were fixed in 10% buffered formalin, dehydrated and embedded in paraffin wax. The embedded tissues were then sectioned at 3-4 μm and stained with haematoxylin and eosin (HE) for microscopic evaluation. For the immunohistochemical investigation of the tissue

sections, monoclonal antibodies for FCoV - Coronavirus pan Monoclonal Antibody FIPV3-70, Invitrogen and macrophages - MAC 387, DAKO were used. Also, DAKO Envision kit based on the Peroxidase/DAB method was used for immunostaining which allowed us to assess the amount and distribution of the FIP antigen. In order to block the endogenous peroxidase, the tissue slides were incubated in 3% H2O2 for 20 min. A citrate buffer pH 6.0 was used by heat treatment in the microwave at 500 W during 20 min as a pretreatment for this method, for antigen retrieval. Then a secondary antibody labelled with horseradish peroxidase (HRP) was added to the slides and they were incubated for 20 min. For the visualization of the reaction, Chromogen, 3.3'- diaminobezidine tetrahidrochloride (DAB - DAKO) was used on the slides and incubated for 5 min at room temperature. Finally, the slides were washed with PBS and stained with Mayers' hematoxylin for contrast.

RESULTS AND DISCUSSION

The gross findings in all cats in this study were as previously described [3,8,9], including: cardiovascular injury, neoplasia, hepatic disease, renal disease, peritonitis, and urinary tract trauma which were the conditions most frequently linked to peritoneal effusion.

The most evident macroscopic post-mortem changes in this study were noted on the intestines in the form of fibrinous polyserositis. The small and large intestine showed various distribution of lymphoplasmacytic infiltrates, in association either with granulomatous/pyogranulomatous lesions (some of them extending through the intestinal wall) or vasculitis which in some cases affected not only the serosa of the intestines, but also the muscularis, submucosa, and the mucosa. The hallmark lesion in our investigation was the presence of pyogranulomas throughout the abdominal serosa with occasional presence of necrotic foci as described [4,10]. Histologically, these formations have central aggregates of macrophages surrounded with neutrophils and macrophages, and to a lesser extent with lymphocytes. Using immunohistochemistry, 10/15 cats were found positive for FIP by detecting FCoV antigen in the intestine.

In the small intestine, solitary mural intestinal lesions i.e. deposits of immune complexes resulting in formation of pyogranuloma are presented (Fig. 1a). Subserosal lesions (arrow) of the small intestine, usually composed of neutrophils and mononuclear cells were present around the vessels. In some lesions, large areas of necrosis (Fig. 1b. N) were also present.

The immunohistochemical staining virus antigens were detected (arrows, Fig. 2a, 2b, 3a, b) within the macrophages (MAC 387 positive cells) within the inflammatory infiltrate around the blood vessels, thus further confirming that the macrophages and monocytes have a major role in the FIV pathogenic as previously described [11].

Although pathognomonic lesions observable under a light microscope are frequently present, to get a conclusive diagnosis of FIP, coronavirus antigens can be identified using immunohistochemistry.

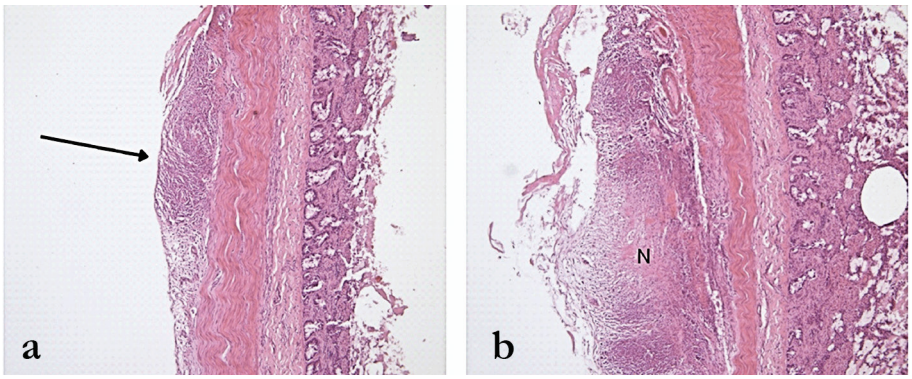

Figure 1. Ileum, **(a)** Pyogranulomatous lesion in the serosa of the small intestine, HE, x 40, **(b)** Large areas of necrosis (N). HE, x 40

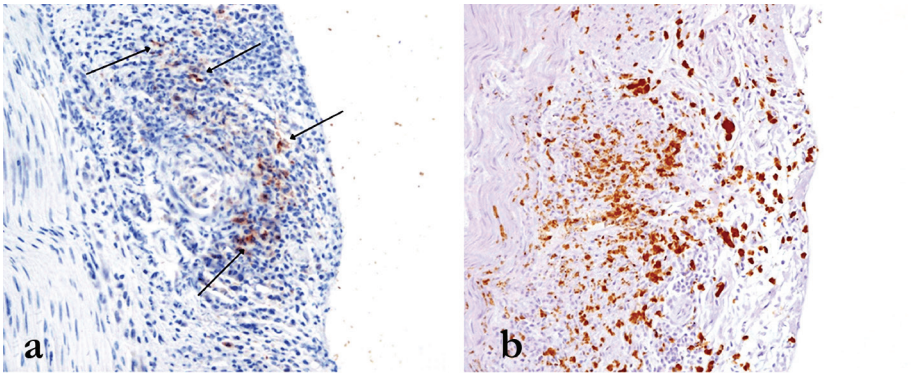

Figure 2. (a) Ileum, FIPV3-70, IHC, x 40, **(b)** Ileum, MAC 387, IHC, x 40

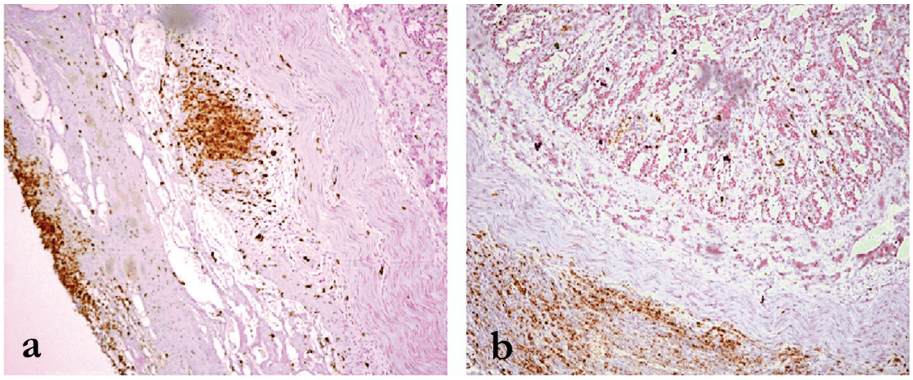

Figure 3. Ileum, **(a, b)** Distribution on the serosa and tunica muscularis, MAC 387, IHC, x 40

The results from the current study revealed ten confirmed FIP cases with FCoV antigen expression in the intestine, while five cats disclosed negative results of clinical investigation suspected on FIP. This confirms the that definitive diagnosis should rely on histopathological examination of tissues and feline coronavirus (FCoV) detection by immunohistochemistry (IHC) as proposed [8,10,11].

CONCLUSION

This study proves that the diagnosis of FIP should be all rounded. The clinical suspicion must be further confirmed using more accurate diagnostic methods such as histopathology and immunohistochemistry. The immunohistochemical method is crucial for a fast and reliable pinpointing of the etiological agent. IHC has better specificity than histopathology, hence it is essential to get several sections of the same tissue in circumstances with strong clinical suspicion, because a negative histopathology result alone cannot rule out FIP. The results from current study revealed ten confirmed FIP cases with FCoV antigen expression in the intestine, while five cats were negative. This confirms that definitive diagnosis should rely on histopathological examination of tissues and feline coronavirus (FCoV) detection by immunohistochemistry (IHC) as proposed by Drechsler et al., Hayashi et al. and Kipar et al. [12,13].

Acknowledgement

This work was supported by the project "Aplication of the immunohistochemical method in the diagnostic of viral diseases in domestic animals", founded by the Faculty of Veterinary Medicine, University of Ss. Cyril and Methodius, Skopje.

Authors' contributions

TN participated in the design, coordination of the study, patient selection and data recordings. IGj, SB and SKK participated in the design of the study, performed the immunohistochemical analysis and helped to draft the manuscript. AJ, EAP and TR participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

REFERENCES

1. Pedersen NC: A review of feline infectious peritonitis virus infection: 1963–2008. J Feline Med Surg 2009, 11(4):225-258.

2. Kipar A, Meli ML: Feline infectious peritonitis: Still an enigma? Vet Pathol 2014, 51(2):505-526.
3. Dewerchin HL, Cornelissen E, Nauwynck HJ: Replication of feline coronaviruses in peripheral blood monocytes. Arch Virol 2005, 150:2483-2500.
4. Stoddart CA, Scott FW: Isolation and identification of feline peritoneal macrophages for *in vitro* studies of coronavirus-macrophage interactions. J Leukoc Biol 1988, 44:319-328.
5. Tasker S: Diagnosis of feline infectious peritonitis: Update on evidence supporting available tests. J Feline Med Surg 2018, 20:228-243.
6. Giori L, Giordano A, Giudice C, Grieco, Paltrinieri S: Performances of different diagnostic tests for feline infectious peritonitis in challenging clinical cases. J Small Anim Pr 2011, 52:152-157.
7. Kipar A, Bellmann S, Kremendahl J, Köhler K, Reinacher M: Cellular composition, coronavirus antigen expression and production of specific antibodies in lesions in feline infectious peritonitis. Vet Immunol Immunopathol 1998, 65(2-4):243-257.
8. Drechsler Y, Alcaraz A, Bossong FJ, Collisson EW, Diniz PPV: Feline Coronavirus in multicat environments. Vet Clin N Am Small Anim Pr 2011, 41:1133-1169.
9. Wright KN, Gompf RE, DeNovo RC Jr: Peritoneal effusion in cats: 65 cases (1981-1997). J Am Vet Med Assoc 1999, 214(3):375-381.
10. Kipar A, Kohler K, Leukert W, Reinacher M: A comparison of lymphatic tissues from cats with spontaneous feline infectious peritonitis (FIP), cats with FIP virus infection but no FIP, and cats with no infection. J Comp Pathol 2001, 125(2-3):182-191.
11. Hayashi T, Watanabe Y, Takenouchi T, Fujiwara K: Role of circulating antibodies in feline infectious peritonitis after oral infection. Jpn J Vet Sci 1983, 45:487-494.
12. Makino H, Hayakawa Ito De Sousa AT, Dandolini Pavelegini LA, Arruda Trevisan YP, Moleta Colodel E, Franco Sousa VR, Dutra V, Nakazato L: Pneumonia in cats associated with *Neisseria* sp. Acta Vet-Beograd 2021, 71(2):211-218.
13. Battilani M, Kaehler E, Tirolo A, Balboni A, Dondi F: Clinicopathological findings in cats tested for Feline Immunodeficiency Virus (FIV) and Feline Leukaemia Virus (FeLV). Acta Vet-Beograd 2022, 72(4):419-432.

IMUNOHISTOHEMIJSKO ISPITIVANJE EKSPRESIJE FIPV3-70 ANTIGENA U ILEUMU MAČAKA SA EFUZIVNIM INFEKTIVNIM PERITONITISOM

Todor NOVAKOV, Ivica GJUROVSKI, Spiro BOZINOSKI,
Aleksandar JANEVSKI, Elena ATANASKOVA PETROV,
Slavica KOSTADINOVA KUNOVSKA, Trpe RISTOSKI

Jedan od najčešćih infektivnih uzroka smrtnosti mačaka je mačji infektivni peritonitis (FIP), zajedno sa panleukopenijom i virusnim infekcijama gornjih disajnih puteva. Veća je verovatnoća da će FIP uticati na mačke čiji je imunski sistem slab ili potisnut. Smatra se da infekcija makrofaga i monocita igra glavnu ulogu u patogenom procesu.

Da bi se postavila konačna dijagnoza ove zarazne bolesti, neophodno je histopatološko ispitivanje tkiva i detekcija mačjeg koronavirusa (FCoV) imunohistohemijom (IHC). U ovom istraživanju, 15 mačaka starosti od 5 do 24 meseca sa kliničkom sumnjom na FIP, podvrgnuto je obdukciji, patohistološkom i imunohistohemijskom pregledu. Rezultati su pokazali da su sve mačke imale abdominalni izliv sa piogranulomima u celoj trbušnoj serozi. Deset od petnaest mačaka je bilo pozitivno na FIP korišćenjem imunohistohemijskih metoda. Ova metoda je takođe pokazala depoziciju antigena u makrofagima, čime je potvrđena njihova uloga u patogenezi FIP-a.