Use of the Immunohistochemical Method in Feline Infectious Peritonitis Diagnosis

ADRIAN STANCU*
Faculty of Veterinary Medicine Timisoara, 119 Calea Aradului, 300645, Timisoara, Romania

PIF was first described by Holzworth in 1963, but is currently reported to be one of the most feared feline diseases in many countries, due to the mortality it can produce, especially among cats of improved breeds and from breeders.

Keywords: peritonitis, infectious, feline, immunohistochemical

PIF Virus (VPIF) is a RNA virus framed in fam. Coronaviridae, the genus Coronavirus. It has the morphology, structure, and biological characteristics of the Coronavirus genus. Within the species Feline infectious peritonitis virus, two types have been identified: the feline infectious peritonitis virus itself (VPIF) and CEF feline enteric coronavirus) which produce the dominant peritonitis symptoms in the cat of enteritis [1-5].

Feline is also susceptible to infection with canine, GET, human and coronavirus coronaviruses, with which it is also related antigenic, but none of them produce peritonitis in cats. Mouse brain, rat, hamster and some feline cell lines are grown with difficulty.

Through the seroneutration reaction, feline coronaviruses were categorized into two serotypes: I and II. Serotype I is prevalent in Europe and is identified in most severe cases of PIF, while serotype II, isolated from more benign forms, is mostly isolated in some Asian countries. Isolated strains exhibit a wide range of degrees of virulence [6-9].

In many bibliographic sources, PIF was described separately from feline enteric coronavirus, as if it were two distinct nosological entities, given the clinical and epidemiological differences between them and even some small differences in the antigenic structure of the viruses. Both entities were referred to as feline coronaviruses [10-16].

Currently, the authors seem to be unanimous in the belief that it is a single morbid entity with two manifestations: a benign one, called feline enteric coronavirus (CEF) and a malignant one called feline infectious peritonitis (PIF). Each of the two clinical forms is the result of infection with one of the two forms of virus existence: the primary form, which is the virus that produces CEF and which is non-pathogenic or poorly virulent, and its virulent mutant that produces PIF [17-22].

Experimental part

Materials and methods

The research was carried out between October 2017 and December 2017 by the necropsisation of seven feline corpses from the Animed Arad Animal Protection Association, which has a collaboration agreement with the Faculty of Veterinary Medicine through the discipline of Forensic Medicine. Necropsy was performed by mammalian specific technique. From the gut, samples were taken to perform the immunohistochemical examination.

The immunohistochemical test reveals viral antigens, nucleocapsid, respectively, in the cytoplasm of infected cells using antibodies obtained on rabbits coupled with immunoperoxidase. Antigen-antibody complexes are visualized at the microscope by means of conjugates made up of the secondary antibody coupled with various chemicals that react with the immunoperoxidase, and the granular granular structures are present in the cytoplasm of the infected cells. Indirect immunoperoxidase assay kit provided by BIO-X Diagnostics was used in the research.

For detection of feline enteric coronavirus (CEF) antigens. (nucleocapsid) present in the infected cells, the kit containing the specific immunoglobulin conjugate coupled to peroxidase was used. For this purpose, intestine was harvested with fibrinous enteric.

From the portion of the intestine, approximately parallelepiped form samples were cut for the working protocol containing 3 parts.

In Part I, each sample was fixed in 4% paraformaldehyde for 24 hours, after which the samples were washed in tap water and held in: 50% alcohol (1 h), 70% alcohol (1 hour) alcohol: 95% (1 h), 100% alcohol (1 h), alcohol: 1: 1 toluene (1 h). The samples were then placed in paraffin I enclosures and held at thermostat at 60°C for two hours and paraffin II kept in a thermostat at 60°C for one hour. The paraffin used had the following composition: 100 g of paraffin + 5 g of wax.

Parts 2 and 3 of this technique were performed according to the Novolink Polymer Detection System (10) immunohistochemical protocol.

In the second part, the blocks were cut into the microtome, the 4 mm thick sections then placed on glass blades, followed by the steps of: dewaxing with toluene (2 baths for 15 min each), rehydration with ethanol (100% 5 min, 96% -5 min, 70% - 5 min), washing the blades with distilled water and removing excess water, neutralizing endogenous peroxidase with PEROXIDASE BLOCK for 10 minutes, washing with TBS 1 5 min), incubation with PROTEIN BLOCK for 10 minutes and washing with TBS 1 (2 bath for 5 min).

In the next step, the conjugate of the primary antibody coupled to the peroxidase in a dilution of 1: 100 was added to the sectional flaps, the flasks being stored in the trays with water in the refrigerator until the next day.

In Part III, the flaps removed from the refrigerator were subjected to the following steps: Wash with TBS 1 (2 baths for 5 min), incubate with POST PRIMARY (30 min), wash with TBS 1 (2 baths for 5 min) and incubating with NOVOLINK POLYMER containing the primary antibody for 30 min, washing with TBS 1 (2 baths for 5 min), treating the blade with 3,3'-diaminobenzidine (DAB) for 5 min, washing with water distilled. Later, hematoxylin was

* email: astancu2002@yahoo.com; Phone: 0722944490

added to the blades as a contrast medium for 40 s. Finally the lamellas were washed with distilled water (2 baths for 5 min) followed by the final washing of the blades with: UNYHOL, UNYHOL PLUS and BIOCLEAR [23-24].

The blades thus prepared were dried, following lamella fastening and microscopic examination.

Results and discussions

In 7 days all cats died. Suspicion of feline infectious peritonitis occurred from the clinical examination of cats when a distension of the abdomen was observed, at which a sensation of sensation was seen on palpation, and at necropsy exsudative inflammation of the serotonin, primarily of the peritoneum. In the serous cavities a viscous, grayish-red light-colored fluid was observed. For the confirmation of the diagnosis, samples were taken from the intestines and the immunohistochemical examination was carried out which showed the fibrinocortico-vascularity. The obtained results confirm the data from the specialized literature (fig. 1, 2).

Natalia Zialkowska et colab. demonstrated in all cases of FIP, fibrinous peritonitis and / or pleuritis with fibrin deposition on serous surfaces. It has revealed a build up of fibrinous fluid, clear to yellowish in the body cavities. Histologically, cats with fluid accumulation in the body cavities were detected in all internal organs examined , multifocal pyrogranulomas consisting of central macrophages, peripheral neutrophils and dispersed plasmid cells. In some mixed-form cats, both the multifocal pig granulomas and the granulomas were observed in the examined organs. Immunohistochemistry showed the presence of FCoV antigens in all organs examined in each case [25].

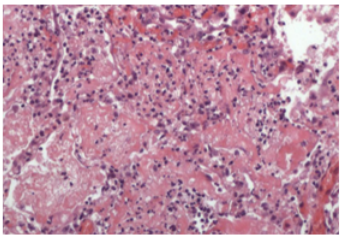

Fig.1. Fibrin-necrotizing vasculitis.

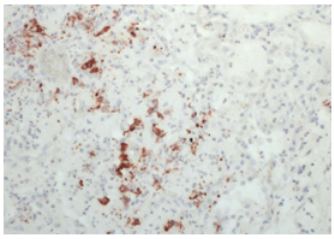

Fig. 2. Viral antigens in intestinal cells highlighted by immunohistochemical examination

Conclusions

In all 7 cases where the FIP test was positive, after the necropsy, lesions of the effusion form were found.

In all 7 cases the exudate was found in both the abdominal cavity and the chest cavity.

Although there are other methods of diagnosing Feline Infectious Peritonitis, the immunohistochemical examination is a sure method of post mortem confirmation of this infectious disease.

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