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Immunohistochemical Diagnosis Method in Parvovirus

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Abstract. *10 cases of histopathologically suggestive lesions for parvovirus in the canine species were examined by the avidin-biotin-peroxidase complex. For this, a mouse monoclonal antibody was used. Following the immunohistochemical examination, positive results were obtained in 80% (8 dogs) of processed samples. The most frequent positive immunohistochemical reaction was observed in macrophages, lymphocytes, necrotic cells of the intestinal crypt, evidence of parvoviral antigen is reliable for determining the etiology of the disease if parvovirus is suspected on the basis of the lesions. In order to perform the diagnostic immunohistochemical procedure, the strepto-avidin-biotin-peroxidase method was used, using the monoclonal antibody. It is of murine origin. Negative cases of parvovirus by immunohistochemical examination show the existence of other causes that produce the same lesions; coronaviruses, rotaviruses or coronaviruses associated with clostridium perfringens which may cause intestinal necrosis of the crypt. All dog breeds can do this disease, but in the Poodle breed the disease has been more common. Sex is not a criterion of receptivity, but in proportion of over 60% were affected females. Regarding the seasonal evolution, the disease is more common in the winter period compared to the other seasons.*

Keywords*: immunohistochimical, diagnosis, dogs, enteric*

1.Introduction

Canine parvovirus is one of the most important and serious diseases of dogs. Since 1978, with the isolation of the virus that produces canine parvovirus, this disease has become much more important.

The disease was discovered on the North American continent, and the causative agent was thought to have arisen from a genetic mutation of the virus that causes panleucopenia in a related species, namely in cats. Shortly, in South America (Mexico), there have been many cases of digestive disorders, characterized by severe gastroenteritis. These cases have been reported in dogs up to one year old [2, 3, 11, 12].

Veterinarians in this country have found a highly contagious disease. Subsequently, in order to elucidate the cause, deep investigations were performed using electron microscopy. The research identified spherical viral particles that had a diameter of 22 nm. Then, by examining 193 serum samples from the previously unvaccinated dog. The purpose was to determine the existence of immunoglobulin against canine parvovirus with the hemagglutination inhibition assay. Following this test, it was found that 17.04% of the samples were positive [2, 11, 15].

In 2003, t presented on the basis of the polymerase chain reaction (PCR) analysis, was able to detect the virus [1, 2, 8, 12, 14].

Immunohistochemical method assists the histopathological diagnosis of several diseases by detecting viral antigens, in tissue sections using specific labeled antibodies, making microscopic binding sites visible; The localization of markers, following the interaction of the antigen antibody, is very important for the detection of proteins, viruses, hormones or even enzymes in a tissue. It is a sensitive method that can detect antibodies that develop against resistant epitopes [4, 9, 17, 18, 20].

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2.Material and methods

The cases from the diagnostic service of the Department of Pathological Anatomy were used. For immunohistochemical investigations, 10 cases from February 2018 to September 2019 were deaths of dogs less than one year old, with suggestive lesions of parvovirosis in the intestinal tissue.

Samples from the small intestine were fixed for 24 h in 10% neutral formalin and processed by paraffin inclusion. Then, the sections obtained were colored according to the method. hematoxylin-eosin then examined under a microscope. Evaluations of all intestinal structures were made:

At the level of mucosa, atrophy of intestinal villi, crypt necrosis, regenerative processes, non-supportive inflammation was observed. At the level of the submucosa, muscle and serous congestion, edema, hemorrhage and inflammatory infiltration.Depending on the degree of impairment, they were classified into mild (one third of the tissue), moderate (two thirds of the tissue) and severe (the entire tissue is affected).

If the atrophy has been observed only in the papillae, we can speak of mild atrophy, and if the intestinal crypts are affected we also speak of severe atrophy.

For detecting viral antigen, the standard immunohistochemical method using strepto-avidin-biotin-peroxidase was used. A canine anti-parvovirus monoclonal antibody of murine origin with a 1/300 dilution was determined. A biotin-labeled secondary anti-mouse antibody was applied and 50 ul was applied to the tissue.

A positive test was used for control, and two negative tests. The first one came from a portion of positive intestinal tissue where the primary antibody was introduced into the PBS solution and for the second control, a portion of intestinal tissue from in a dog that had no parvovirus lesions, to which a primary antibody was added.

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Depending on the degree of impairment, they were classified into mild (1/3 of the tissue), medium affected (2/3 of the tissue) and severe forms (includes the entire structure). When the lesions have been observed only at the tip of the papillae, it is low atrophy, and when the crypts are completely affected it is of severe form. The standard strepto-avidin-biotin-peroxidase immunohistochemical method was used to detect viral antigen.

A monoclonal antibody from a murine canine antiparvovirus origin was determined under dilution at 1/300. A secondary goat anti-mouse antibody, biotin-labeled, was applied and applied 50 µL to the tissue.

The immunohistochemical technique was used for the positive control test. For this, a portion of the small intestine was used.

Two negative control tests were also used. For one, positive tissue samples were used, for the identification of the primary antibody, a PBS solution was used, and for the other control, a subtitle intestine was used from a patient who had no parvovirus lesions, for whom the primary antibody was used. were processed according to the paraffin technique, sectioned at 6 µm.

For tissue rehydration, the blades were submerged in ethyl alcohol in decreasing concentrations starting from 100% alcohol; then 96%, 80% and finally 50% alcohol. The washing process was followed for a period of 5 min, in the case of each individual alcohol concentration. Endogenous peroxidase inhibition was performed using hydrogen peroxide and methanol, being introduced twice,

for 30 min each. Then, the next wash in PBS solution. Each wash took 5 min and was performed at room temperature.

For antigen retrieval, tissues from PBSthey were put into physical treatment in a microwave oven over 4 minutes. Then, the hot PBS was changed to stop the cold PBSrecovery reaction.In order to block avidinand endogenous tissue biotin, a regularitythe blocker was used with two solutions.

The tissues were coated with two drops of 50 µL / drop of avidin, then incubated at 25°C, 45 min, followed by 3 successive washes in PBS. Then the tissues were covered with goat nonimmune serum (2 drops), and kept for 60 min at room temperature. Then each blade was coated with 2 drops of the primary antibody in preparation. They were also incubated for 12 h at room temperature. Two controls were followed, at the first replacement of the primary antibody, for the next control, the same dilution of the antibody was kept, followed by incubation and washing three times for 5 min with PBS solution.

A secondary goat antibody was also used, of which 2 drops were used to cover the blades, then they were kept under similar conditions of temperature and humidity for 90 min.

Three successive washes were performed, for 5 min each, in PBS solution in order to remove the excess secondary antibody.

After preparation and use of the chromogen, samples were introduced into Meyer hematoxylin. Then the samples were introduced into the PBS solution for 30s. The positive reaction was characterized by the presence of intracellular granules [5, 7, 17].

3.Results and discussions

In the small intestine, epithelial cells in the glandular crypts matureand migrate from the germinal epithelium to the tip. Then, the epithelial cells acquirethe absorption capacity of the nutrients. CPV-2 infects the germinal epithelium in the intestinal crypts and causes destruction of the epithelium. Histopathological aspects in intestines from dogs positive to parvoviral infection are characterized by necrosis of glandular crypts, presence of mononuclear infiltrate inflammatory, atrophy and fusion of intestinal villi. These issues were also encountered by other researchers, who describe similar lesions depending on the severity of the infection.

Although there are several studies that report atrophies and intranuclear inclusions in cryptelot glandular cells, this study was not observed. It has been observed that at the level of the submucosa, lymphoid atrophy is frequently encountered, which coincides in the literature. The immuno-histochemical examination confirmed over 80% positive cases, compared to the total number of cases examined. This percentage is lower compared to the studies performed by Ku et al. [19] et al.

The immunohistochemical method used was usual, using strepto-avidin-biotin-peroxidase and a monoclonal antibody of murine origin. In more recent studies, Svara et al. applied nonhistochemical technique on intestinal sections and other organs of 20 dogs with a clinician and topatological diagnosis of CPV-2 with strepto-avidin-biotin-peroxidase complex with polyclonal antibody. The positivity was detected in 95% of the intestines and in 88.24% of different organs: liver, kidney, spleen, thymus, lymph nodes and lungs; was not found in the heart. In the epithelial cells and in the glandular intestinal crypt, both in the nucleus and in the cytoplasm [4]. In this study, necrotic cells andinflammatory cells present the main number of positive cases, this coincides with Svara et al. In our study, a correlation between the localization of the antigen and the histopathological structures was observed, namely, a high degree of positivity was found in the low intensity histopathological lesions, and a low degree of positivity in the severe lesions [16].

Obvservations by Mc Cartney et al. who studied cases between the ages of 8 and 14 weeks, which were infected orally. The degree of cell regeneration was considered as an index of the presence of canine parvovirus. Miura et al. considered that the presence of these cells is characteristic in CPV-2 infection, as well as the presence of necrosis of intestinal crypts and inclusion bodies [6,8].

There were lesions associated with non-specific virus.: hyperplasia of the glandular crypts, hyperplasia of the caliciform cells, the inflammatory suppurative influx haemorrhage, edema and colonies of bacteria, always bearing in mind that these lesions may be present in other digestive, pathologies.

Negative cases of parvovirus infection by immunohistochemical examination may suggest the involvement of other etiological agents of a viral or bacterial nature, which cause lesions identical to that of; coronaviruses, rotaviruses or coronaviruses associated with clostridium perfringens, which can cause necrosis of intestinal crypts. All breeds can be affected by this disease, but it is an observer who can make the breed Poodle disease incidence has been increased. Over 60% of countries are affected by females. The disease can occur in any season but is often the largest it is winter.

Aspects related to the incidence of immunopositive lesions in infection with CPV-2 in canine parvovirus and eloquent lesions are shown in Table 1, respectively Figures 1 and 2.

Table 1. Frequency of histopatological injuries in the case of positive reactions in the canin parvovirus frombowel

Structural changes	Nomber of dog	Low	Average	High
INTESTINAL MUCOSA				
Atrophy of intestinal villi	8	2	4	2
Necrosis of cript	8	1	5	2
Non supurative inflammation	8	5	2	1
Superficial necrosis	5	3	3	2
Congestion	4	5	3	0
Hemorrhages	2	1	1	0
Repair	3	1	1	0
Edema	2	1	1	0
Hyperplasia of crypts	1	1	0	0
Hyperplasia of globet cells	1	1	0	0
Inclusion bodies	0	0	0	0
SUBMUCOSA				
Edema	6	3	2	1
Non supurative inflammation	3	2	1	0
Congestion	6	3	3	0
Hemorrhages	2	2	0	0
Repair	1	1	0	0
MUSCULAR				
Non supurative inflammation	2	1	1	0
Eedma	6	4	2	0
Congestion	3	3	0	0
Hemorrhages	2	1	1	0
SEROSA				
Non supurative inflammation	3	3	0	0
Congestion	2	2	0	0
Hemorrhages	1	2	0	0
Edema	3	2	1	0

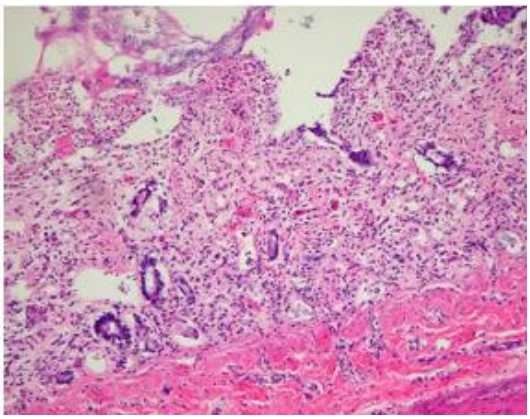

Figure 1. Intestine of dog. Histophatological aspect. Necrosis/loss of intestinal crypt. Col H.E. Parvovirosis

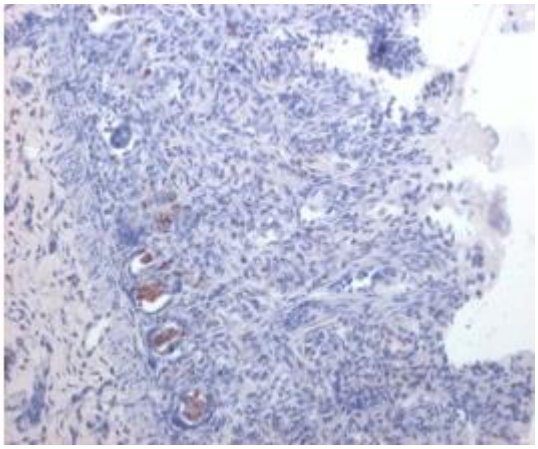

Figure 2. Intestine of dog. Immunohistochimical positiv aspect in intestinal crypts. Col H.E. Parvovirosis 100X

4.Conclusions

Immunohistochemical technique revealed the presence of parvovirus, after most of the cases were previously suspected by Parvovirosis following the histopathological examination.

The percentage was lower than the data provided by the literature, obtained by some authors.

Cellular regeneration was the lesion that was correlated with canine parvovirus infection, and was observed using immunohistochemical technique.

Because there are viral diseases that cause lesions in the intestine, it would be necessary to implement some differential diagnosis methods.

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