Macedonian Veterinary Review
Mac Vet Rev 2023; 46 (2): 193-198
sciendo
Available online at
www.macvetrev.mk

Short Communication

IMMUNOHISTOCHEMICAL DIAGNOSTIC CHARACTERISTICS OF PARVOVIRUS INFECTION IN DOGS

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Received 10 February 2023; Received in revised form 21 June 2023; Accepted 10 July 2023

ABSTRACT

The current study goal was to compare the results from the histopathological and immunohistochemical findings in dogs that have previously been clinically diagnosed with parvovirus infection. The Canine parvovirus enteritis (PVE) is a highly contagious disease mostly affecting dogs below six months of age. The canine parvovirus (CPV) belongs to the parvoviridae family within the feline parvovirus sub group of the genus Parvovirus. This investigation was performed on twenty dogs 2 to 12 months old with previous clinical diagnosis for PVE, and it included necropsy, histopathology, and immunohistochemistry. The necropsy findings included severe hemorrhagic enteritis and enlarged mesenteric lymph nodes as most frequent and prominent changes. The histopathological changes were also most significant in these organs in the form of villus atrophy and lymphoid depletion, respectively. These areas revealed the highest expression of CPV antigen. The histopathological and immunohistochemical methods provide strong base for a reliable CPV diagnosis.

Key words: diagnosis, necropsy, histopathology, immunohistochemistry, canine parvovirus

INTRODUCTION

Canine parvovirus enteritis (PVE) is a highly contagious disease (1) being observed for the first time in the late 1970s as a worldwide infectious disease mostly affecting puppies (2). The causative agent belongs to the parvoviridae family (1). Canine parvovirus (CPV) is almost identical to feline panleukopenia virus (FPLV), as it is within the feline parvovirus sub group of the genus Parvovirus (3). Currently, there are three familiar strains of canine parvovirus throughout the world: CPV-2a, CPV-2b, and CPV-2c. All ages of dogs have been shown to be susceptible to CPV, with the highest deathrate of 67.6% in dogs below six months of age and declining with the rise in age (4). The disease is prevalent in unvaccinated dogs. The high susceptibility in puppies is due to lack of immunity from maternally derived antibodies, unsuitable time of vaccination, and/or from ineffective responses to vaccinations (5).

The major route of transmission between dogs is indirect, via the fecal-oral route (6, 7). However, the virus can also be transmitted directly (8). There are two clinically distinguished canine parvoviral forms: gastroenteritis in puppies 6-20 weeks of age which happens in the period when the maternal antibody protection declines and vaccination has not yet produced enough protection against infection (9, 10), and myocarditis in young puppies, especially in the early neonatal period (8). Usual clinical findings of PVE infection include vomiting,

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Competing Interests: The authors have declared that no competing interests exist.
Available Online First: 24 July 2023
Published on: 15 October 2023
https://doi.org/10.2478/macvetrev-2023-0024

bloody diarrhea with specific odor, dehydration, anorexia, hypovolemic shock, hypothermia or fever, marked thrombocytopenia and leucopenia, as well as tachycardia, and hypotension (11). Palpation reveals abdominal pain due to acute gastroenteritis or intussusceptions (12).

Postmortem examination reveals changes characteristic for PVE. They include small intestine enteritis with segmental distribution and usually affecting jejunum and ileum but not duodenum and colon. Mesenteric lymph nodes may be enlarged and edematous with multifocal petechial hemorrhages in the cortex (13, 14, 15). In case of parvoviral myocarditis, there is dilatation of the left atrium and ventricle. The lungs are not collapsed. Microscopically, there is necrosis of epithelial cells in the intestinal crypts and severe lymphocyte depletion in the lymphoid tissue. In the tongue, small intestine, and Payer's patches, intra-nuclear inclusion bodies are usually present (12). These findings are the basis for the histological diagnosis of PVE in dogs (16, 17, 18, 19, 20). Fast and accurate diagnosis of PVE is essential in order to stop the spreading of infection among dogs. The clinical signs are not exclusive to PVE so a confirmation with laboratory tests is necessary (1). This is usually done by various laboratory methods that detect canine parvovirus in the feces of infected dogs such as immunochromatographic tests (IC), electron microscopy (EM), hemagglutination inhibition (HI) tests, Enzyme-linked immunosorbent assay (ELISA), conventional and real-time polymerase chain reaction (C-PCR), and real-time PCR (RTPCR) (21). However, for the postmortem examination, the finding of characteristic lesions as well as presence of antigen and/or nucleic acid within those lesions is a criterion suggested by many as reliable in many viral diseases (22, 23). Immunohistochemical (IHC) staining of formalin-fixed tissues for parvovirus is a sensitive and specific method for demonstrating viral antigen in tissue sections even before histologic lesions are evident (24).

The goal of the study was to compare the results from the immunohistochemical and pathohistological methods in the diagnosis of the Canine parvovirus enteritis (PVE).

MATERIAL AND METHODS

Animals

This study was conveyed *post mortem* on twenty dogs at age between 2 and 12 months in the span of a two-year period. All of the dogs had previously been clinically diagnosed as positive for CPV. The severity of the parvovirus symptoms ranged from vomiting and bloody diarrhea with specific odor followed by dehydration and hypovolemic shock.

All dogs were submitted for analyses with verbal consent of the owners. A complete necropsy was performed on all of the dogs immediately after the animal's death, during which the morphological changes of the internal organs were observed and also tissue samples including the small and large intestine, lungs, mesenteric lymph nodes, and spleen were collected for further investigation.

Histopathology and immunohistochemistry

The tissue samples collected at necropsy were routinely fixated in 10% buffered formalin for 48-72 hours, dehydrated and embedded in paraffin wax, and afterwards cut at 3-4 μm thick slides. In order to evaluate the tissue changes and to establish the possible correlation with the PVE, the slides were stained with hematoxylin and eosin (HE) and microscopically examined. The immunohistochemical investigation was performed as described by Gjurovski et al. (25). A canine/feline parvovirus (CPV1-2A1) monoclonal antibody was used along with DAKO Envision kit based on the Peroxidase/DAB method. This method allowed for an insight in the amount and distribution of the CPV antigen within the tissue and its correlation with the damaged areas. The tissue slides were incubated in 3% H_2O_2 for 20 min in order to block the endogenous peroxidase. The pretreatment for antigen retrieval comprised of citrate buffer pH 6.0 by heat treatment in microwave at 500 W for 20 min. The slides were incubated overnight with the parvovirus antibody. The slides were later added a secondary antibody labelled with horseradish peroxidase (HRP) and were incubated for 20 min. To allow a visualization of the reaction, a Chromogen, 3.3'- diaminobezidine tetrahidrochloride (DAB - DAKO) was added. The process ended by washing the slides with PBS and staining them with Mayers' hematoxylin for contrasting. Normal rabbit sera were used for the treatment of control tissue sections.

RESULTS

Gross findings

All dogs were anemic and cachectic with bloody feces around their anus. Severe hemorrhagic enteritis was the hallmark finding in all of them. The small intestinal mucosa was hyperemic and

edematous. The stomach mucosa was hyperemic. The Payer's patches, the mesenteric lymph nodes and the spleen were enlarged.

Histopathology

Catarrhal to hemorrhagic enteritis with multifocal crypt necrosis and destruction of crypt intestinal epithelial cells within the small intestine was observed in all animals. Twelve dogs had hemorrhagic enteritis, while eight of them had severe catarrhal enteritis. The intestinal villi were atrophied (Fig. 2). The lumen of the intestines contained large amount of epithelial cell debris as well as inflammatory cells. Lymphocyte infiltration was observed in the mucosa of the small intestine accompanied by the presence of some neutrophils. Some of the samples had hyperplastic gland cells. Lymphoid depletion was evident in the lymph nodes (Fig. 1). The histological findings are presented in Table 1.

Immunohistochemistry

The immunohistochemical staining in this investigation showed that the distribution of the viral antigen was mostly present in the mesenteric lymph nodes of the dogs (Fig. 3). It was also detected in significant amount in the jejunum and the ileum, whereas the duodenum and colon were negative. The antigen distribution within the lymph nodes was mostly visible in the lymphocytes (Fig. 3). In the small intestine, the antigen was located in the epithelial cells of the villi and crypts (Fig. 4). No positive reaction was observed in the other tissues and in control sections. The distribution of PCV2 antigen in the areas with alterations is presented in Table 2.

Table 1. Distribution of histopathological findings in the jejunum and ileum of 20 puppies

Histopathological change	Number of findings
Hyperemia	20/20
Lymphocyte infiltrate	20/20
Atrophy of villi	14/20
Cell debris in the intestinal lumen	17/20
Desquamation and hyperplasia of glands	15/20
Hemorrhages	12/20

Table 2. Distribution of PCV2 antigen in the altered areas

Tissue	Degree of alteration	Distribution of PCV2 antigen
Intestinal villi	+++	++
Payer's patches	++	++
Mesenteric lymph nodes	++	+++
Lungs	+	+

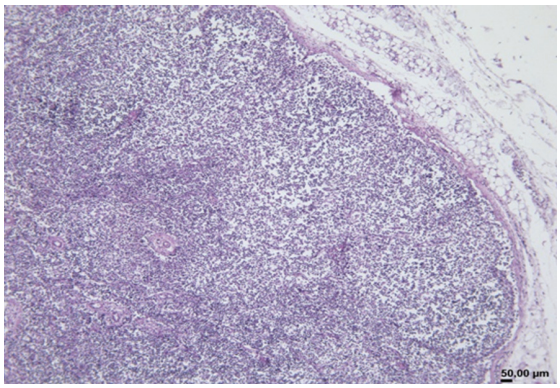

Figure 1. Lymph node: Lymphoid depletion HE, X 100

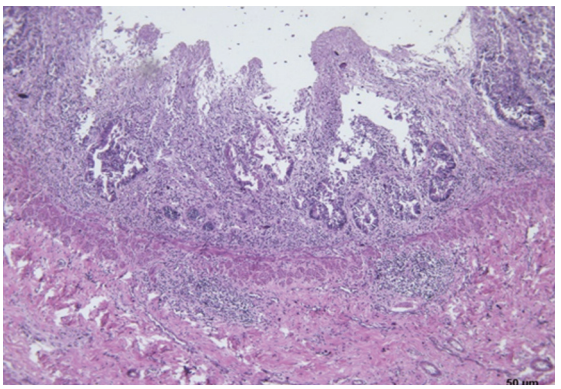

Figure 2. Small intestine: villus atrophy and necrosis of intestinal glands, HE, X 100

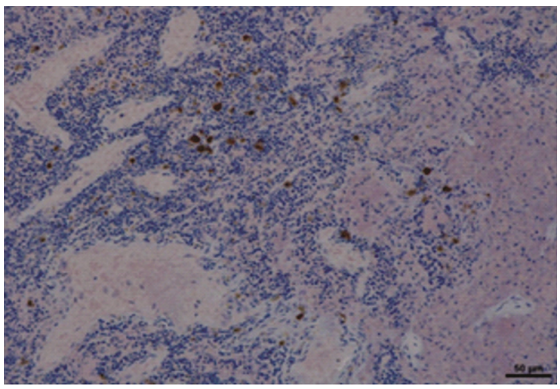

Figure 3. Lymph node: Parvoviral antigens in lymphocytes. IHC, X 200

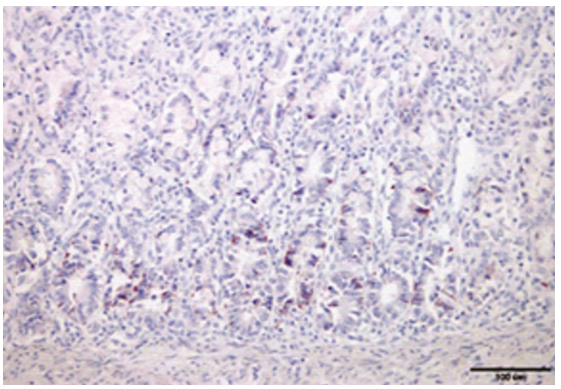

Figure 4. Small intestine: PVE antigen in the enterocytes. IHC, X 200

DISCUSSION

At present the diagnosis of PVE is based on clinical, pathological, virological, and serological examinations (26, 27, 28, 29, 30, 31). The clinical signs in PVE are not pathognomonic and a final diagnosis cannot be determined based on them. Methods for diagnosis of PVE such as serological or fecal examination cannot differentiate an illness as the virus can be present in healthy animals (26, 32). The IHC is a suitable method in cases where pathological signs may not yet be developed in all cases.

This article presented the postmortem histopathological and immunohistochemical findings and made comparison between the two in twenty dogs previously diagnosed or suspected of PVE on the basis of clinical symptoms. The results clearly suggest a correlation between the presence of the CPV antigen and the degree of histopathological lesions in the examined tissues. The accent of the investigation was set on the small intestine, mesenteric lymph nodes, as well as the parenchymal organs such as lungs and spleen. Most significant are the changes affecting the small intestine and the lymphoid tissue. The changes found in them included villus atrophy in the jejunum and ileum and necrosis of Payer's patches. These findings are characteristic but not definite findings for PVE as suggested by Evermann at al. (30) and Berkin et al. (32).

The immunohistochemical method provided a direct insight in the presence and the distribution of the CPV antigen in the tissues. According to Dogonyaro (1), Gombac et al. (4), and Decaro et al. (7), the antigen distribution corelates with the organ's mitotic activity. In this investigation, the detection of PVE was the highest in the jejunum and ileum, which are organs with the highest mitotic activity. The results show that the degree of lymphoid depletion correlates with the amount of CPV antigen in the affected organs. The results provided in this study suggest that the histopathological and the immunohistochemical method can provide a reliable diagnosis for the canine parvovirus infection.

CONCLUSION

The results from the presented study suggest that the observed tissue damage along with the

CPV antigen distribution within them is a relevant method for diagnosis of the parvoviral diseases in dogs. In order to improve the diagnostic accuracy, the immunohistochemical method should be included especially when there are inconclusive clinical and pathomorphological findings.

CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

ACKNOWLEDGMENTS

This work was supported by the project "Aplication of the immunohistochemical method in the diagnostic of virus diseases in domestic animals" funded by the Faculty of Veterinary Medicine - Skopje, Ss. Cyril and Methodius University in Skopje (FVMS-IPR-03).

AUTHORS' CONTRIBUTIONS

IG and TR wrote the article. SB and EM collected the samples. SB, TN, MFB and TR analized the samples. All authors approved the final version of the study.

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Please cite this article as: Gjurovski I., Bozinovski S., Murdzevska E., Novakov T., Bozkurt M.F., Ristoski T. Immunohistochemical diagnostic characteristics of parvovirus infection in dogs. Mac Vet Rev 2023; 46 (2): 193-198. https://doi.org/10.2478/macvetrev-2023-0024