Acta Scientiae Veterinariae, 2024. 52: 1964.

RESEARCH ARTICLE
Pub. 1964

ISSN 1679-9216

Canine Parvoviral Enteritis - The Role of MMP-9 and TIMP-1 in the Pathogenesis of Intestinal Inflammation

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ABSTRACT

***Background*:** Canine parvoviral enteritis is a highly contagious infection in the intestines caused great morbidity and mortality in untreated dogs younger than 6 months. Matrix metalloproteinases consist of zinc- and calcium-dependent extracellular matrix-degrading endopeptidases that are tightly controlled by endogenous metalloproteinase tissue inhibitors. Canine parvoviral enteritis is common in Turkey. The aim of this study was to examine the expression of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) in natural canine parvoviral enteritis infection of 25 dogs diagnosed with parvoviral enteritis by clinical tests and histopathology.

***Materials, Methods & Results*:** The study material consists of dog's small intestine, which was brought to Cumhuriyet University Faculty of Veterinary Medicine pathology department for necropsy and diagnosed with parvoviral enteritis. This investigation was suported by the Comission of Scientific Research Projects of Cumhuriyet University (Project No: V-086). For the study, sections of 5 µm were taken from small intestine blocks consisting of duodenum, jejunum and ileum, fixed in 10% buffered formalin solution and embedded in paraffin, and stained with hematoxylin-eosin and MMP-9 and TIMP-1 antibodies using immunohistochemical procedure. On histopathology, shedding and blunting of the villi epithelium, severe mononuclear inflammation in the lamina propria and locally enlarged crypts with lymphocytolysis in peyer's patches were noted in the ileum. Immunohistochemically, strong expression for MMP-9 and moderate expression for TIMP-1 were observed in the crypt epithelium and inflammatory cells in the small intestines of infected animals compared controls ($P < 0.001$).

***Discussion*:** In the present study, immunohistochemical expressions of MMP-9 and TIMP-1 in intestinal tissues were investigated in canine parvoviral enteritis, which is an important viral disease in veterinary medicine. Statistically strong expression for MMP-9 and moderate expression for TIMP-1 were observed in the crypt epithelium and inflammatory cells in the small intestines of infected animals. As a result, high levels of MMP-9 may be one of the factors that trigger the inflammatory process in the disease. It is thought that the increase in MMP-9 may be directly proportional to the severity of inflammation in the tissue. In addition, it is suggested that the level of its inhibitor, TIMP-1, may increase at similar rates in response to this increase in MMP-9 levels. As a result, severe increases MMP-9 and TIMP-1 may indicate the presence of inflammation of similar severity in that tissue. Immunohistochemical data obtained from the study showed that MMP-9 expression was found to increase in inflammatory and degenerative changes in parvoviral enteritis. This may have triggered extracellular matrix degradation, intestinal permeability, degenerative changes and inflammation. Abnormal increase in MMP-9 levels is thought to contribute significantly to the intestinal lesions in parvoviral enteritis. It was observed that TIMP-1 levels increased similarly in response to this increase but weaker expression of TIMP-1 as its inhibitor in canine parvoviral enteritis may determine the development of the disease. In this regard, matrix metalloproteinases appear to be potential therapeutic targets in canine parvoviral enteritis, and the use of their inhibitors can significantly reduce disease progression. However, current findings need to be confirmed by more detailed studies in the future.

Keywords: dog, viral infection, metalloproteinase activity, MMP-9, parvoviral enteritis, TIMP-1.

DOI: 10.22456/1679-9216.141400

Received: 12 June 2024 Accepted: 27 September 2024 Published: 18 October 2024

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INTRODUCTION

Canine parvoviral enteritis is a very common infectious disease that usually affects dogs younger than 6 months [2,9,19]. After the virus is ingested by the digestive tract, it prefers cells with high mitotic activity in the digestive tract and bone marrow, then causes severe anorexia, vomiting, foul smelling diarrhea, fever, dehydration and weakness clinically [24].

Matrix metalloproteinases (MMPs) consist of zinc- and calcium-dependent extracellular matrix-degrading endopeptidases that are tightly controlled by endogenous metalloproteinase tissue inhibitors [4,5,17]. MMPs play an important role in embryonic development, differentiation, proliferation and tissue regeneration [17]. Typically, little or no MMP expression occurs in normal healthy tissues, but intense expression can occur in some diseases, tissue repair process, or inflammation [25]. MMP-9 plays an important role in degranulating extracellular matrix proteins and shaping tissue remodelling [3,13,22,25]. Metalloproteinase activity is regulated by complex activators, cell receptors and inhibitors, particularly tissue inhibitor of matrix metalloproteinases (TIMPs) [18]. TIMP-1 potentially stimulates cell proliferation and stabilizes cells against apoptotic stimuli [5]. TIMPs can inhibit or sometimes enable MMPs' activity [10]. Together with its endogenous inhibitor TIMP-1, MMPs regulate signaling pathways that are very important in inflammation [3].

The aim of this study is to investigate the simultaneous expression of MMP-9 and TIMP-1, which has not been investigated before, in the pathogenesis of natural canine parvoviral enteritis infection.

MATERIALS AND METHODS

Animals

In the study, 25 dog's intestinal tissues, brought to Cumhuriyet University Faculty of Veterinary Medicine, Department of Pathology for necropsy and diagnosed with parvo viral enteritis by clinical (IDEXX, SNAP Parvo Test)[1] and histopathological methods, were used. A total of 10 non-lesional intestinal tissues of dogs that have died from other diseases were used as negative controls. For the study, sections of 5 μm were taken from small intestine blocks consisting of duodenum, jejunum and ileum, fixed in 10% buffered formalin solution and embedded in paraffin, and stained with Hematoxylin-Eosin (HE)[2] using a routine procedure for light microscopy.

Immunohistochemical (IHC) staining was performed using the routine streptavidinbiotin-peroxidase technique according to the manufacturer's recommendations [Anti rabbit streptoavidin/biotin immunoperoxidase kit (Histostain-Plus Kits)][3]. The selected 5 μm paraffin tissue sections were stained immunohistochemically in order to elucidate the expression of MMP-9 [anti-MMP-9 antibody, Biorbyt (diluted 1/250)][4] and TIMP-1 [anti-TIMP-1 antibody, Biorbyt, (diluted 1/250)][4]. Color reaction was enhanced using 3-amino-9-ethylcarbazole (AEC) (Zymed AEC RED substrat kit, ABD)[5] as the chromogen. All sections were counterstained with Gill hematoxylin (Merck, Germany)[2] solution and then washed in water. Coverslips were applied with water-based mounting medium. Stained sections were examined under a light microscope (Nikon, YS 100)[6].

Immunohistochemical analysis

Immunolabeling of sections with the MMP-9 and TIMP-1 antibodies were scored semiquantitatively. An area with a high density of positively labeled cells was selected. The percentage of cells labeled were evaluated at X200 final magnification using an eyepiece with grids of 100 squares from 10 adjacent fields that constituted a total area of 0.050 mm^2. The percentages of labeled cells in the lamina propria were scored as follows: (–) no staining (negative), (+) area of stained cells <10% (weak staining), (++) area of stained cells =10- 30% (moderate staining), (+++) area of stained cells >30% (strong staining).

Statistics analysis

Statistical analysis was performed using the Mann Whitney U test in SPSS 26 program. $P < 0.001$ was considered statistically significant. Statistical analysis of the data on MMP-9 and TIMP-1 expressions in intestinal tissue, measured by immunostaining in all the groups, are listed in Table 1.

Table 1. Statistical analysis of MMP-9 and TIMP-1 immunohistochemical staining in Parvovirus infection and control groups.

Group	MMP-9	Timp-1
	Mean (Median) ± SEM	Mean (Median) ± SEM
Parvovirus infection	2.72 (3.00) ± 0.11***	2.16 (2.00) ± 0.13***
Control	0.40 (0.00) ± 0.16	0.30 (0.00) ± 0.15

RESULTS

In the study, small intestinal tissue sections of 25 dogs were diagnosed with parvoviral enteritis by clinical tests and they were evaluated histopathologically. According to this evaluation, shedding and blunting of the villi epithelium, hyperplasia of the crypt epithelium, severe mononuclear inflammation in the lamina propria and locally enlarged crypts with lymphocytolysis in peyer's patches were seen in all animals especially in the ileum (Figure 1)

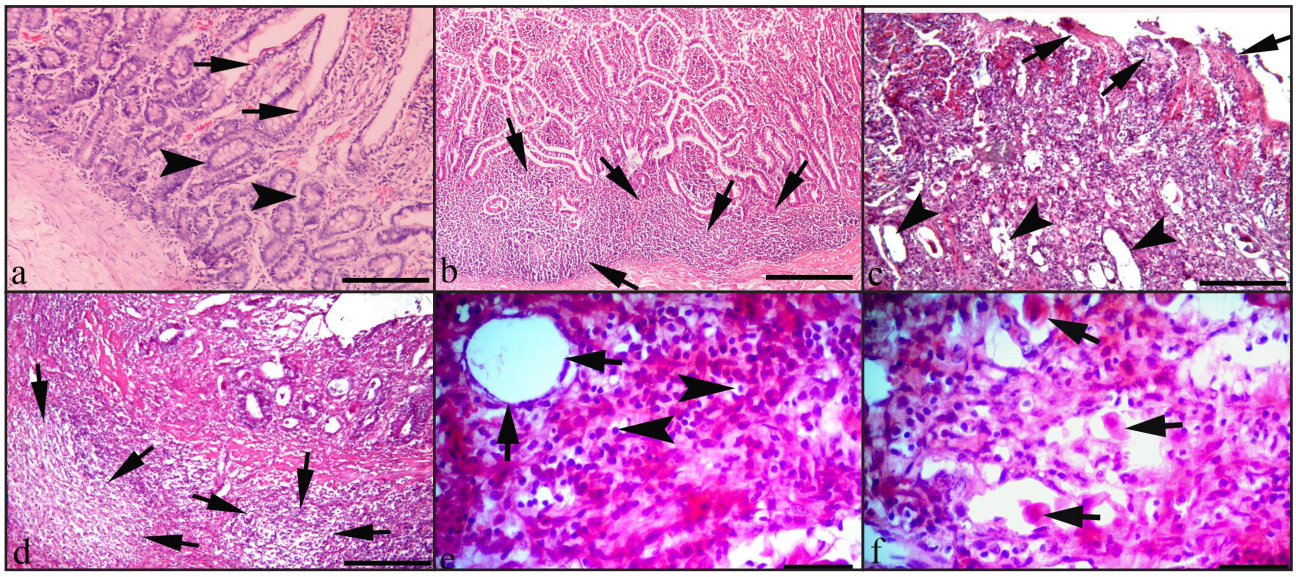

Figure 1. Histopathological examination of intestine tissues with parvoviral enteritis. a- Intact villi epithelium (arrows) and crypts (arrowheads) in control intestinal tissue. [HE; Bar=100 µm]. b- Normal Peyer's patches in control tissue (arrows). [HE; Bar=100 µm]. c- Desquamation of epithelial villi (arrows) and dilatation and degeneration in the crypts (arrowheads). Parvoviral enteritis. [HE; Bar=100 µm]. d- Lymphocytolysis in Peyer's patches (arrows). Parvoviral enteritis. [HE; Bar=100 µm]. e- Dilatation in the crypts (arrows) and mononuclear cell infiltration in the lamina propria (arrowheads). Parvoviral enteritis. [HE; Bar=25 µm]. f- Desquame crypt epithelium (arrows). Parvoviral enteritis. [HE; Bar=25 µm].

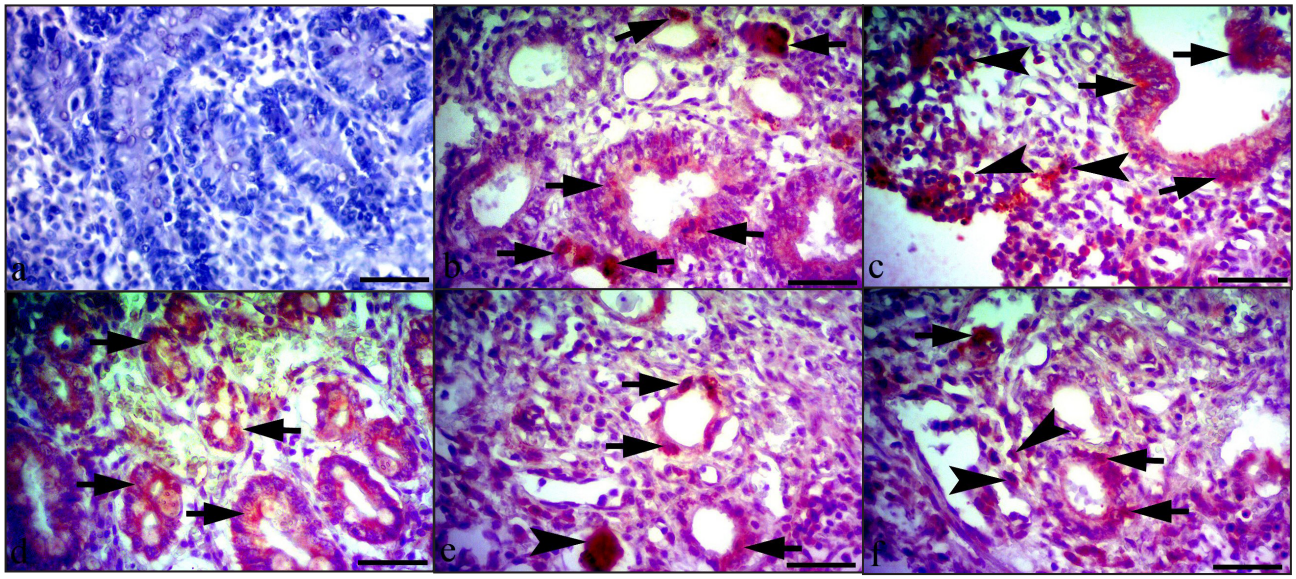

Figure 2. MMP-9 expression from intestinal tissues with control and parvoviral enteritis. a- Control group. [IHC; Bar=25 µm]. b- Strong cytoplasmic immunoreactivity for MMP-9 in dilate crypt epithelium (arrows). Parvoviral enteritis. [IHC; Bar=25 µm]. c- Strong MMP-9 immunopositive inflammatory cells in the lamina propria (arrowheads) and MMP-9 expression in dilated and hyperplastic crypt epithelium (arrows). Parvoviral enteritis. [IHC; Bar=25 µm]. d- MM-9 expression from crypt epithelium (arrows). Parvoviral enteritis. [IHC; Bar=25 µm]. e- MMP-9 immunostaining in dilate cript epithelium (arrows) and desquame cells in lumen (arrowhead). Parvoviral enteritis. [IHC; Bar=25 µm]. f- Strong MMP-9 immunopositive inflamatory cells in the lamina propria (arrowheads) and MMP-9 expression from degenerative crypt epithelium (arrows). Parvoviral enteritis. [IHC; Bar=25 µm].

In the immunohistochemical staining, cytoplasmic MMP-9 expression detected as strong, especially from the crypt epithelium and inflammatory mononuclear cells in the small intestines of infected animals (Figure 2). Immunostaining of TIMP-1 was observed moderate staining in the cytoplasm of the crypt epithelium and inflammatory cells in the small intestines of infected animals (Figure 3). Weak immunohistochemical expression was obtained for both antibodies in control animals. Immunohistochemical analysis showed significant up-regulation of MMP-9 (Figure 4) and TIMP-1 (Figure 5) expression in parvoviral enteritis when compared to controls ($P < 0.001$).

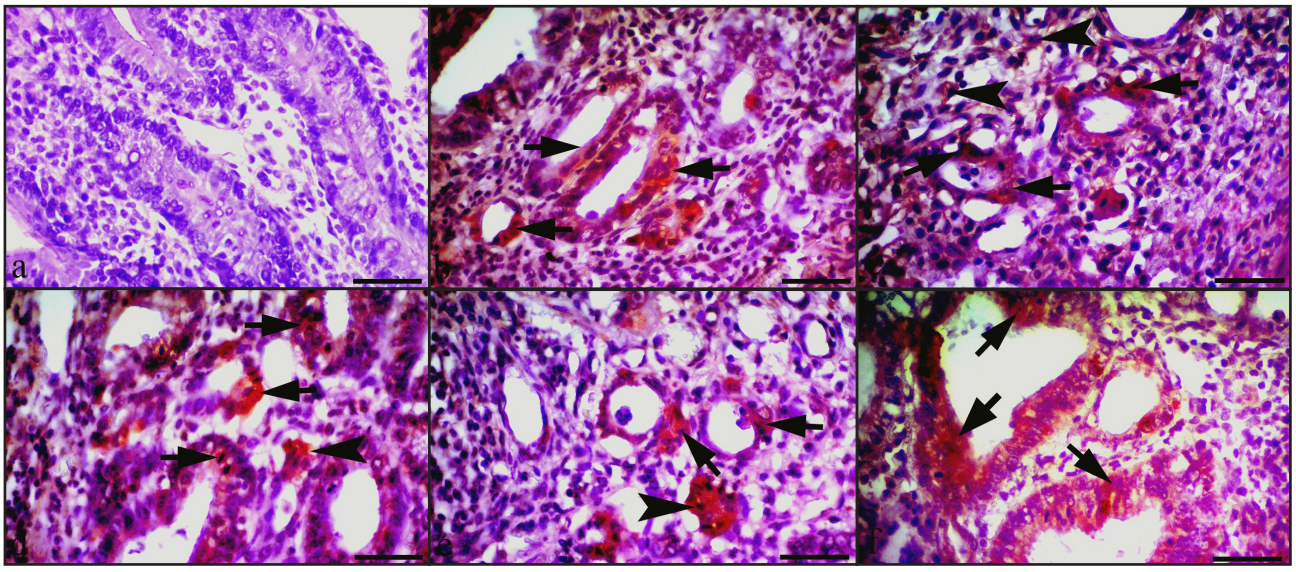

Figure 3. TIMP-1 expression from intestinal tissues with control and parvoviral enteritis. a- Control group. [IHC; Bar=25 µm]. b- Cytoplasmic TIMP-1 expression in crypt epithelium (arrows). Parvoviral enteritis. [IHC; Bar=25 µm]. c- Cytoplasmic TIMP-1 expression in crypt epithelium (arrows) and inflamatory cells (arrow heads) in lamina propria. Parvoviral enteritis. [IHC; Bar=25 µm]. d & e- Cytoplasmic TIMP-1 immunopositivity in dilate (arrows) and hyperplastic crypt epithelial cells (arrow head). Parvoviral enteritis. [IHC; Bar=25 µm]. f- Cytoplasmic TIMP-1 immunopositivity in dilate and hyperplastic crypt epithelial cells (arrows). Parvoviral enteritis. [IHC; Bar=25 µm].

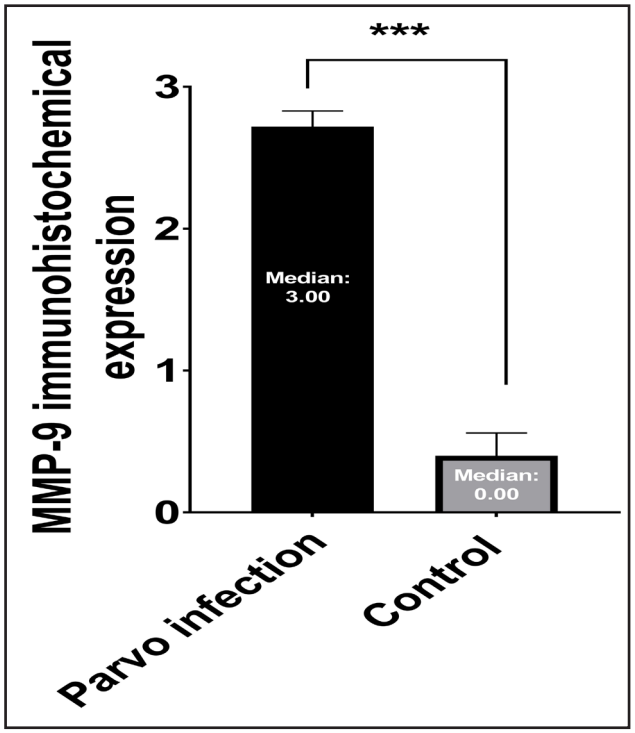

Figure 4. Comparison of MMP-9 expression intensity in control and parvoviral infection.

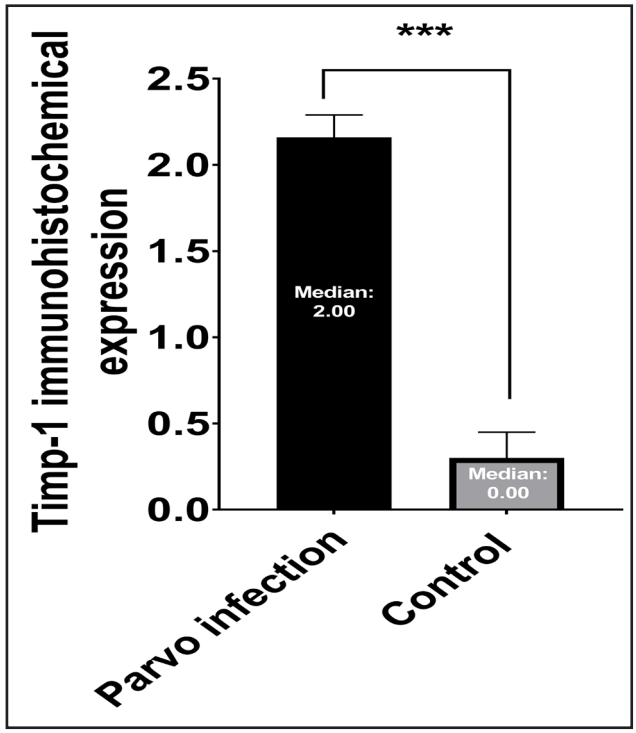

Figure 5. Comparison of TIMP-1 expression intensity in control and parvoviral infection.

DISCUSSION

MMPs are released from fibroblasts, epithelial cells, and some inflammatory cells in association with infectious intestinal inflammations [20,22]. Abnormal regulation of MMPs is a significant factor in many diseases and pathological conditions such as inflammatory processes, tissue destruction, fibrosis, abnormal angiogenesis, autoimmune diseases and carcinogenesis [1]. There are limited articles on the role of MMP-9 in animal diseases. MMP-9 is unique as its protein expression and activity is undetectable in most healthy intestinal tissues but has been shown to be highly expressed in a variety of inflammatory states, including inflammatory bowel disease [21]. Recently, it has been reported that MMP-9 causes an increase in the tight junction permeability of the intestinal epithelium. The intestinal epithelium is a single layer of cells within the gut lumen that serves an important protective function. This structure is a selectively permeable barrier that allows the absorption of nutrients but prevents harmful luminal contents from crossing the intestinal epithelium [6,8]. This barrier damage, resulting in increased intestinal permeability, contributes significantly to the pathogenesis of inflammatory bowel disease, including Crohn's disease, ulcerative colitis, celiac disease, and other inflammatory conditions in the gut [11,16]. In this study, statistically significant increase in MMP-9 expression was observed in animals with parvoviral enteritis when compared to controls ($P < 0.001$). It suggests that the increase in MMP-9 expression may have impaired the tight junction permeability of the intestinal epithelium in the pathogenesis of parvoviral enteritis. In addition, it is thought that inflammation increases with ECM destruction and interleukin activation. [12,15] and as a result, the clinical findings are exacerbated. In the study, parvoviral enteritis in dogs was diagnosed by focal antigen, clinical symptoms, histopathology and haematological findings. The dogs with parvoviral enteritis and control were examined for Giardia, Cryptosporidium and coccidiosis, and those with negative results were included in the study. Even if there is another unidentified disease in a few animals that may contribute to intestinal lesions, it is thought that the results of the study will not change significantly with the statistics performed on 25 animals.

MMPs are inhibited by TIMPs, which are endogenous protein regulators and tissue inhibitors [1,23]. Inside the ECM, TIMPs inhibit the proteolytic activity of MMPs. In this context, a disruption or alteration in the balance between MMPs and TIMPs plays a role in the pathophysiology and progression of various diseases [7]. Previous study suggests that MMP-2, MMP-7, and MMP-9 may be a potential therapeutic target, and the use of inhibitors of these such as TIMP-1 and TIMP-2 can significantly decrease ulcerative colitis improvement in human [14]. In the present study, strong MMP-9 and moderate TIMP-1 expression were observed in the crypt epithelium and inflammatory cells in the small intestines of infected animals ($P < 0.001$). These results suggest that increase in MMP 9 may be directly proportional to the severity of inflammation occurring in the tissue. In addition, it was thought that TIMP-1 levels increased at a similar rate in order to prevent the increase in MMP-9 levels. As a result, increased levels of MMP-9 and TIMP-1 may indicate the presence of inflammation of similar severity in that tissue.

CONCLUSSION

Immunohistochemical data obtained from the study showed that MMP-9 expression was found to increase in inflammatory and degenerative changes in parvoviral enteritis. This may have triggered ECM degradation, intestinal permeability, degenerative changes and inflammation. Abnormal increase in MMP-9 levels is thought to contribute significantly to the intestinal lesions in parvoviral enteritis. It was observed that TIMP-1 levels increased similarly in response to this increase but weaker expression of TIMP-1 as its inhibitor in canine parvoviral enteritis disease may determine the development of the disease. In this regard, MMPs appear to be potential therapeutic targets in canine parvoviral enteritis, and the use of their inhibitors can significantly reduce disease progression. However, current findings need to be confirmed by more detailed studies in the future.

MANUFACTURERS

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Funding. This study was suported by the Comission of Scientific Research Projects of Cumhuriyet University (Project No: V-086).

Ethical approval. Approval by the Committee on the Ethics of Animal Experiments of the Cumhuriyet University was not required because the experiment did not involve any invasive procedures for animal experiment and none of the animals was sacrificed.

Declaration of interest. The authors have declared no conflict of interests. The authors alone are responsible for the content and writing of the paper.

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