Histopathological and immunohistological findings in canine parvoviral infection: diagnosis application

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SUMMARY

In this study, 10 dog puppies, 2 - 12 months old, from different breeds, were used for the comparative evaluation of histopathological findings observed in canine parvoviral infection with immunohistological detection of parvovirus antigens. Dogs exhibited diarrhoea, vomiting and cachexia and after necropsy and histopathological examination, severe enteritis (catarrhal enteritis in 8 cases, haemorrhagic and fibrinous enteritis in 2 cases) was evidenced. Although the intra-nuclear inclusion bodies, pathognomonic of the parvoviral infection, were not observed, some other characteristic signs such as villus atrophy and/or necrosis of the Payer’s patches or fibrinous / haemorrhagic enteritis at a lesser extend, were found in 6 cases. The immunohistological detection of viral antigens was also positive for a total of 6 puppies in jejunum / ileum and mesenteric lymph nodes (6 cases), in adrenal glands (3 cases) and also in lungs (1 case). Nevertheless, animals (n = 2) with haemorrhagic and fibrinous enteritis were found negative whereas 2 dogs displaying none of the above histopathological mentioned signs were found positive. These results indicate that in the absence of the pathognomonic sign of the disease, characteristic histopathological signs may not always allow diagnosis and that immunohistochimistry may enhance the diagnosis value.

Keywords: Dog, parvoviral enteritis, immunohistochemistry, ileum, jejunum, villus atrophy.

RÉSUMÉ

Dans cette étude, 10 chiots de même âge, âgés de 2 à 12 mois, provenant de races différentes, ont été utilisés afin de comparer les données histopathologiques observées lors de parvovirose avec la détection immunohistochimique des antigènes viraux. Les chiots, cachectiques, présentaient de la diarrhée et des vomissements et après autopsie, une entérite sévère a été mise en évidence à l’examen histopathologique de type catarrhal dans 8 cas et de type fibrineux et/ou hémorragique dans 2 cas. Bien que les incluions nucléaires pathognomoniques de la parvovirose n’aient pas été décelées, d’autres signes caractéristiques tels que l’atrophie des villosités et/ou des plaques de Payer nécrotiques et une entérite fibrineuse ou hémorragique à un moindre degré, ont été observés dans 6 cas. La détection immunohistochimique des antigènes viraux s’est avérée positive pour 6 chiots aussi, les particules virales étant localisées dans le jéjunum, l’iléon et les nœuds lymphatiques mésentériques pour les 6 cas, dans les surrénales dans 3 cas et dans les poumons dans un des 6 cas. Néanmoins, les animaux (n = 2) ayant une entérite hémorragique ou fibrineuse ont donné un résultat négatif alors que 2 chiots ne présentant aucun des signes histologiques caractéristiques ont donné un résultat positif à l’immunohistochimie. Ces résultats montrent qu’en l’absence de signe pathognomonique de la maladie, les données histopathologiques ne sont pas toujours suffisantes et que l’immunohistochimie apporte une plus-value dans l’établissement du diagnostic.

Mots clés : Chien, entérite parvovirale, immunohistochemie, iléon, jéjunum, atrophie des villosités.

Introduction

The canine parvovirus (CPV) has been reported in many countries around the world. In Turkey, the presence of this disease was first reported in 1981 [4]. Two clinical forms of the disease, mainly observed in young dogs, are identified: the cardiac and the enteric forms. The cardiac form occurs mainly in early ages and is associated with a not purulent interstitial myocarditis. The enteric form is more frequently encountered, preferentially in older animals and is associated with catarrhal, haemorrhagic and fibrinous inflammation [4, 10, 11, 13, 17, 21, 23, 24]. Fatal enteritis due to the parvovirus infection is still a fairly common disease in dogs despite use of vaccines. Additionally, necrosis and atrophy of the bone marrow, lymphoid tissue and thymus as well as interstitial pneumonia and multiform erythema were reported [5, 8, 19]. Another typical effect of the virus is hypoplasia of the cerebellum and encephalitis in new-born animals due to intrauterine infection [1, 13, 27]. Especially, histological diagnosis is based on the presence of classical lesions such as loss of intestinal epithelium, necrosis of lymphoid tissues and demonstration of inclusion bodies [3-5, 8, 10, 11, 17, 19, 21] that are not always seen in the intestine and heart. On the other hand, both clinical and histological findings are resem-bled to those in cells infected with different viruses (e.g. rotavirus and coronavirus) [22]. In the context, certain confirmation of the aetiology can only be achieved by the viral detection (e.g. viral nucleic acid or antigen) in the lesions [30].

In this study, pathological findings in the CPV infection were evaluated according to the clinical anamnesis, and the diagnostic value was compared to that of a diagnostic immunohistological method based on the viral detection in tissues.
Materials and Methods

ANIMALS

Four female and six male unvaccinated dogs, 2 - 12 months old, were brought to the clinics of the Faculty of Veterinary Medicine, Ankara, with the complaints of diarrhoea, vomiting and cachexia. This dog population consisted in 6 cross-breeds, 2 rottweilers, 1 collie and 1 kangal. After death, necropsies were performed and tissue samples (thymus, heart, lung, stomach and intestinal segments, mesenteric lymph nodes, adrenal gland, kidney, liver, spleen, bone marrow and central nervous system) were immediately collected and fixed in 10% buffered formalin.

HISTOLOGICAL AND IMMUNOHISTOLOGICAL ANALYSES

The tissue samples were processed in compliance with the standard paraffin wax technique. The 5 μm thick sections were stained with the haematoxylin-eosin stain [15].

The immunohistochemistry (Avidin-Biotin Complex Peroxidase (ABC-P)/Cadenza Tags-Shandon) was performed according to the manufacturer’s instructions. Briefly, after dewaxing and rehydration, endogenous peroxidase activity in tissue sections was blocked by applying 0.3% hydrogen peroxide in methanol for 20 minutes at 37°C and thereafter samples were treated with pronase for 10 minutes at 37°C. After incubation with normal goat sera for 20 minutes at 37°C, sections were incubated with the monoclonal primary antibody anti-canine parvovirus antibody diluted to 1:600 (Lo-Imex, University of Louvain, Brussels, Belgium) for 1 hour at 37°C. Subsequently, the biotinylated goat anti-rabbit IgG were added for 1 hour at 37°C following by the streptavidin-peroxidase reagent for 20 minutes at 37°C. The colour was developed by a final incubation with 3-amino-9 ethyl carbazole (AEC) for 5 minutes at room temperature. After each incubation step, the sections were thoroughly washed with phosphate buffered saline (PBS) solution, excluding the step performed with normal goat sera. The sections were counterstained with Gill’s Haematoxylin. The tissue sections treated with normal rabbit sera served as controls. Additionally, the samples were also taken from the intestines and mesenteric lymph nodes of all the animals for microbiological examination.

Results

MACROSCOPIC FINDINGS

Dehydration, anaemia (evidenced by pale mucosa) and cachexia were observed in all animals. The peri-anal region was contaminated with faeces in three animals, one with tinges of blood. Ten to fifteen mL of a serous yellow fluid was present in the abdominal cavity in 2 dogs.

In 9 dogs, yellowish, watery, and partly mucous faeces were found in the lumens of small intestines and were associated with hyperaemic and oedematous mucosa. Furthermore, slight petechial haemorrhages were observed in the mucosa of the duodenum in one animal. Payer’s patches were evident. In almost all animals, severe enteritis was diagnosed. The jejunum and ileum were thickened and had a pipe-like appearance (figure 1). Bloody and mucoid content was present in the stomach, which the mucosa was highly hyperaemic with few haemorrhagic foci (0.5 cm in diameter) (figure 2). Mesenteric lymph nodes were enlarged and pale. In the last puppy (3.5 month old), watery and bloody faeces were observed in the intestinal lumen and petechial haemorrhages were mainly localised in the duodenum and jejunum, and displayed a linear pattern around the ileo-caecal valve.

MICROSCOPIC FINDINGS

Abundant sloughed epithelial and inflammatory cells as well as cell debris were found in the lumens of intestines. Hyperaemia of the mucosa infiltrated by lymphocytes and some neutrophils, denudation and distortion of the villi (figure 3) were also observed. A mild proliferation of the connective tissue was observed in the propria mucosa and sub-mucosa. Desquamation and secretion accumulation were present in the lumen of some intestinal glands. Hyperplastic gland cells and syncytial formations were recorded in 4 animals. Hyperplasia in the Payer’s patches leading to necrosis was observed in all animals. Consequently, the diagnosis of catarhal enteritis was proposed in 8 cases (Table I). In one animal diagnosed with haemorrhagic enteritis, numerous erythrocytes were present in the intestinal lumen and wide areas of haemorrhages in the propria mucosa and partly between the sub-mucosa and the tunica muscularis were also evidenced. In the last puppy, similar but more intense findings were observed and the diagnosis of fibrinous enteritis was established. There was a moderate lymphadenitis in the mesenteric lymph nodes.

In addition, an E. coli strain of unknown pathogenecity was isolated from the case of fibrinous enteritis.

IMMUNOHISTOLOGICAL FINDINGS

The viral antigens were detected in the jejunum and ileum parts of the small intestines and in mesenteric lymph nodes in 6 cases, in the adrenal glands in 3 cases and in the lungs in one case (Table II). They had an intracytoplasmic location in the form of fine or coarse, reddish-brown granules in the epithelial cells covering the tips of dilated and atrophic villi, crypts and propria of the jejunum and ileum (figures 4 and 5). A similar reaction was detected in lymphocytes located in the propria mucosa (figure 6) and from mesenteric lymph nodes (figure 7). No immunostaining was evidenced in the duodenum and in the colon. In the lungs, positive reaction corresponding to intracytoplasmic coarse granules or diffuse staining in some alveolar cells (figure 8) was restricted in few dispersed areas. In the adrenal gland, the viral particles exhibited both cytoplasmic and intra-nuclear localisation.

No positive reaction was observed in the other tissues and in control sections.
CANINE PARVOVIROSIS: HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

TABLE I: Frequency of the histological findings observed in the small intestines from puppies with spontaneous parvoviral infection (n = 10).

<table>
<thead>
<tr>
<th>Site</th>
<th>Histological events</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen</td>
<td>Cell debris / epithelial and inflammatory cells</td>
<td>8/10</td>
</tr>
<tr>
<td></td>
<td>Erythrocytes</td>
<td>2/10</td>
</tr>
<tr>
<td>Mucosa</td>
<td>Atrophy of villi</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>Gland modifications</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td>Desquamation</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td>Hyperplasia and syncitia formation</td>
<td>4/10</td>
</tr>
<tr>
<td></td>
<td>Hyperaemia</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Haemorrhages</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte infiltrate</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td>Hyperplasia or necrosis of the Payer’s patches</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Connective tissue proliferation</td>
<td>1/10</td>
</tr>
<tr>
<td>Mesenteric nodes</td>
<td>Inflammation</td>
<td>10/10</td>
</tr>
<tr>
<td>Histological diagnosis</td>
<td>Catarrhal enteritis</td>
<td>8/10</td>
</tr>
<tr>
<td></td>
<td>Haemorrhagic enteritis</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>Fibrinous enteritis</td>
<td>1/10</td>
</tr>
</tbody>
</table>

Figure 1: Fibrin (arrows) and haemorrhage (arrowhead) in small intestine from a 3.5 month old puppy with parvoviral infection.

Figure 2: Haemorrhages in stomach (arrows) from a 8 month old puppy with parvoviral infection.

Figure 3: Partial atrophy of villi (arrow) and necrosis (n) of the Payer’s patches in the jejunum from a 4.5 month old puppy with parvoviral infection. Haematoxylin - eosin, X 100.

Figure 4: Parvoviral antigens in cytoplasm of some epithelial cells (arrows) from ileum villi in a 4.5 month old puppy with parvoviral infection. Avidin-Biotin Complex Peroxidase (ABC-P) method, X 250.
Discussion

The canine parvoviral enteritis diagnosis is based on clinical, pathological, virological and serological examinations [2, 7, 8, 12, 18, 26, 27]. Serological tests are generally used to confirm the presence or the passage of the infection within a population and they cannot provide a definite diagnostic for a given subject. The viral detection in faecal suspensions by electron microscopic examination directly confirms the presence of the virus in the organism [18] but this method also presents some drawbacks because the virus may also be detected in the faeces of healthy animals [2, 6]. Clinical signs, like in the present study, may not provide adequate diagnostic information and only lead to a possibility of parvoviral infection. Therefore, other methods should be employed for an accurate diagnosis [9, 22].
Histopathologically, some inclusion bodies are formed in the intestinal cells at the end of the incubation period before the occurrence of clinical signs. Since clinical signs develop due to the cellular destruction, the diagnostic histological evidence gradually disappears. Although these bodies may be seen in few remaining cells, they may be easily confused with the cell nuclei [24]. Subsequently, the inclusion bodies are not considered as a major element for the final diagnosis [3, 24]. In the current study, the absence of such inclusion bodies even in dogs for which the presence of viral antigen was confirmed by immunohistochemistry further supports this view. On the other hand, the villus atrophy in the jejunum and ileum and the necrosis of Payer’s patches were considered as two characteristic findings [4, 7, 9, 20, 28].

It has been emphasized that diagnosis may suffer from confusion with other viral and bacterial infections, primarily corona- and rotavirus infections [3, 7, 14, 25] which are often diagnosed after CPV infection, in 41% and 6% of cases respectively [6, 28]. However, in the present study, neither of these two histological findings was detected in 3 cases, one displaying a subacute course and a second exhibiting a fibrinous enteritis. In these animals, which were not diagnosed with infection according to the morphological examination, viral antigens were also not detected by immunohistochemistry. The villus atrophy and the necrosis of the Payer’s patches were found in one haemorrhagic and in two acute cattural enteritis cases whereas in two acute cattural enteritis cases only one of the two findings was detected. Nevertheless, the animal diagnosed with haemorrhagic enteritis (dog n°2) was found negative by immunohistological examination. The difference between immunohistological and pathological findings was also evident in 2 other animals (dogs n°1 and 7): although none of the typical histological criteria (villus atrophy and necrosis of Payer’s patches) was evidenced in these 2 dogs, they have given immunopositive staining of viral antigens in the jejunum and ileum epithelial cells, in the mesenteric lymph nodes and even (dog n°7) in the adrenal glands. As pathological signs may not clearly develop in all cases, the immunohistochemical method may be proposed for a reliable diagnosis.

The presence of the viral antigens revealed by immunohistochemistry in the small intestines (jejunum and ileum) was attributed to the destruction of cells in which viral agents were heavily present before the development of clinical signs. The presence of viral antigens in a small number of cells from other organs and tissues including the myocardium [1, 16, 31] was detected in some of the animals. It was admitted that the detection of the viral agent decreases in parallel to the decrease of the mitotic activity in organs and tissue cells with the age [29]. Consequently, the virus immunolabelling would be maximal in tissues with a high mitotic activity such as jejunum and ileum particularly in young animals. Furthermore, MACARTNEY and MACARTNEY [16] claimed that the intra-nuclear localisation of the parvoviral antigens was more relevant for diagnostic than the intracytoplasmic localisation. In the present study, intra-nuclear staining was only recorded for infected lymphocytes from the intestinal lymph follicles and mesenteric lymph nodes. Although BERGMANN et al. [3] and FRESE and REINACHER [9] reported intra-nuclear location of the viral antigens in epithelial cells covering the surface of intestinal crypts and villi, they have also observed intracytoplasmic locations of the viruses in cells from the same region and even extracellular viral elements in the crypt lumens. The cytoplasmic position of the viral antigens was also observed in other organs and tissues [8, 16, 28].

The results of the present study showed that, in the canine parvoviral enteritis, as long as the pathognomonic histopathological findings i.e. inclusion bodies are not detected, characteristic pathological findings do not suffice for diagnosis. Since immunohistological methods are considered to be more reliable, when both methods are consulted, the diagnosis of especially suspected cases would be guaranteed. In addition, the immunohistological methods also allow the identification of other enteropathogens known to cause similar morphological findings and contribute to a differential diagnosis.

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References


