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Case report

Naturally occurring systemic canine distemper virus infection in a pup

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Abstract

This report describes the pathologic and immunohistochemical findings associated with naturally occurring systemic canine distemper virus (CDV) infection in a dog. Clinically, there was lymphopenia and hyperkeratosis of footpads, while pneumonia was identified by radiology. Gross lesions consisted of bilateral ocular discharge and discrete digital hyperkeratosis. By histopathology, there was severe interstitial pneumonia and necrosis of splenic germinal centers. Additionally, eosinophilic intranuclear inclusion bodies were observed within cerebellar astrocytes without marked manifestation of CDV-induced encephalitis. Further, intracytoplasmic bodies were identified within the epithelial cells of the renal pelvis, lung, and urinary bladder. Immunohistochemistry identified the presence of CDV antigens in these tissues and further confirmed the systemic infection to the epidermis of footpads, heart, liver, intestine, spleen, and lymph node.

Key Words: dog; canine distemper virus, histopathology, immunohistochemistry.

Introduction

Canine distemper virus (CDV) infection is a fatal disease that produces severe neuropathological syndromes and systemic infection in dogs. Young pups, immature dogs not adequately vaccinated or without adequate protection by maternal antibodies are more susceptible to infection (1, 6). Canine distemper in dogs is well controlled in Finland by vaccination and there have been no detailed pathological descriptions of this disease published after the last severe outbreak of distemper in 1994-1995 (5). Further, only 2.85% (19/666) of samples from dogs evaluated during 1996-2007 in Finland by laboratory methods were confirmed as CDV positive (14). Therefore, this report describes the pathological and immunohistochemical findings of systemic CDV infection in a young dog, since this infection is important in countries with low prevalence of the disease, especially in dogs with inadequate vaccination history or susceptible immunosuppressive state.

Case report

A three-month-old, male, Boxer dog arrived at the Veterinary Teaching Hospital, University of Helsinki, Finland, with a history of anorexia, marked reduction in body weight, and dyspnea during a two-week period. The owner reported that the animal had been in contact with a dog from Russia; the vaccination history was unknown. Clinically, hyperkeratosis of footpads and dehydration were diagnosed. Laboratory examination revealed leukopenia (2.15 x 10^9/l; normal 5.4-17.4 x 10^9/l), severe thrombocytopenia (25.3 x 10^9/l; normal 102-395 x 10^9/l), lymphopenia (0.1 x 10^9/l; normal 1-5.4 x 10^9/l), and neutropenia (1.9 x 10^9/l; normal 2.9-13.8 x 10^9/l). Abnormalities were also observed within the population of red blood cells (RBC); having reduced values of total RBCs (3.21 x 10^{12}/l; normal 5.3-8 x 10^{12}/l), hematocrit (20.6%; normal, 37-35%), and hemoglobin concentration

(66.7g/l; normal, 140-203 g/l). Additionally, there were alterations to the mean corpuscular volume (64.2 fl; normal 67-80 fl), the mean corpuscular hemoglobin (20.8 pg; normal 24-29 pg), and the mean corpuscular hemoglobin concentration (323 g/l; normal 345-367 g/l). Further, pneumonia was identified by radiologic analysis. The animal was euthanized due to poor prognosis; routine necropsy was performed soon after death.

Grossly, there was moderate bilateral purulent ocular secretion and discrete hyperkeratosis of the footpads of the thoracic and pelvic members (Fig. 1A); the lungs were non-collapsible with discrete to moderate increase in consistency. Other significant gross alterations were not observed. Tissues (brain, liver, lung, heart, lymph node, kidneys, urinary bladder, spleen, and skin from the footpads) were fixed in 10% neutral-buffered formalin solution and routinely processed for histopathologic evaluation; selected formalin-fixed paraffin-embedded sections were submitted for immunohistochemistry (IHC) using commercially produced monoclonal antibody for CDV (Serotec, Kidlington, Oxford, UK) and canine parvovirus (CPV, Serotec). IHC was done by using the streptavidin-biotin technique performed in an automated detection system (LabVision, California, USA). Tissue from a previous case of canine distemper served as positive control for distemper (10), while that from an unpublished case of canine parvoviral enteritis was used for CPV.

Significant histological alterations were observed in the lungs, urinary bladder, spleen, and footpads. Pulmonary lesions were characterized by diffuse severe interstitial pneumonia associated with numerous intracytoplasmic eosinophilic inclusion bodies within epithelial cells (Fig. 1B). There was severe degeneration of the transitional epithelial cells of the urinary bladder, and many of these dilated cells contained intracytoplasmic inclusion bodies (Fig. 1C). Footpad lesions consisted of discrete hyperkeratosis and epithelial hyperplasia with moderate multifocal degeneration of keratinocytes of the stratum spinosum layer (Fig. 1D). Splenic alteration was manifested as severe necrosis of germinal centers (Fig. 1E). Neurological alterations were discrete; consisted predominantly of moderate perivascular influx of lymphoplasmacytic inflammatory cells at the choroid plexuses, discrete neuronal necrosis, and discrete vacuolization of the cerebellar white matter associated with several intranuclear eosinophilic inclusion bodies in astrocytes (Fig. 1F). Intestinal lesions consisted of discrete dilation and necrosis of few intestinal crypts (Fig. 1G). Intracytoplasmic inclusion bodies were observed within the epithelial cells of the renal pelvis (Fig. 1H) without any related lesion to the renal parenchyma. Additionally, there were areas of discrete degeneration of cardiac muscle fibers and severe depletion of lymphoid tissue of the lymph nodes.

CDV antigens were easily identified within the epithelial cells of bronchi and bronchioles, vascular endothelium, alveolar epithelial cells, and within alveolar macrophages of the lung by immunohistochemistry (Fig. 2A). Although histological brain lesions were mild, there were severe accumulations of CDV antigens within astrocytes, and within the epithelial cells of the choroid plexus, ependyma, vascular endothelium and meninges of the cerebellum (Fig. 2B-C). Several vascular endothelial cells and neurons of the cerebral cortex contained CDV antigens (Fig. 2D). Positive CDV immunostaining was observed within the epithelial cells of the lamina propria of the intestine, the epithelial cells adjacent to necrotic intestinal crypts, and vascular endothelium of the submucosa and serosal layers (Fig. 2E). The lymphocytes around necrotic splenic germinal centers were intensely positive for viral antigens (Fig. 2F); this was also observed in lymphocytes throughout the affected lymph nodes (Fig. 2G). Within the kidney, positive CDV antigens were observed within vascular endothelial cells and the epithelial cells of the renal pelvis (Fig 2H). The transitional epithelium of the urinary bladder was severely stained positive for CDV antigens (Fig. 3A). Additionally, cardiac muscle fibers (Fig. 3B), epithelial cells of bile ducts, vascular endothelial cells, and macrophages of the liver (Fig. 3C) were positive for CDV antigens. Although distinct inclusion bodies were not observed within the footpad epidermis, IHC revealed that keratinocytes, fibroblasts, and the epithelial cells of sebaceous glands, vascular endothelium, and hair follicles contained CDV antigens (Fig. 3D). Intestinal tissues reacted negatively to antigens of CPV.

The histopathological lesions and immunohistochemical findings observed in this case are characteristic of systemic infection induced by CDV in a pup without adequate immunity (1, 6), and indicate that vaccination must be intensified to maintain Finland free of distemper. Vaccination is recommended at 3, 4, and 12 months of age, with additional boosters every two years thereafter (5, 14). Therefore, in some cases, vaccination strategies might also be extended to include the gravid bitch so that maternal antibodies can be passively transferred to the suckling pup. Further, more than 90% of the passive transfer of CDV antibody occurs via colostrum, and is maintained up to approximately 12-14 weeks of age (6). The adequate vaccination of the mother of this dog might have prevented the occurrence of this infection in the case herein described due to the passive transfer of immunization via colostrum. Further, maternal transference of antibody to young pups that have not yet been immunized by routine vaccination is a good preventative vaccination strategy (6).

In this case, systemic canine distemper infection was initially diagnosed due to the characteristic histopathological lesions associated with typical eosinophilic inclusion bodies (intranuclear and intracytoplasmic) within epithelial cells of the pulmonary, nervous, cutaneous, and urinary systems; the presence of CDV in these tissues was then confirmed by

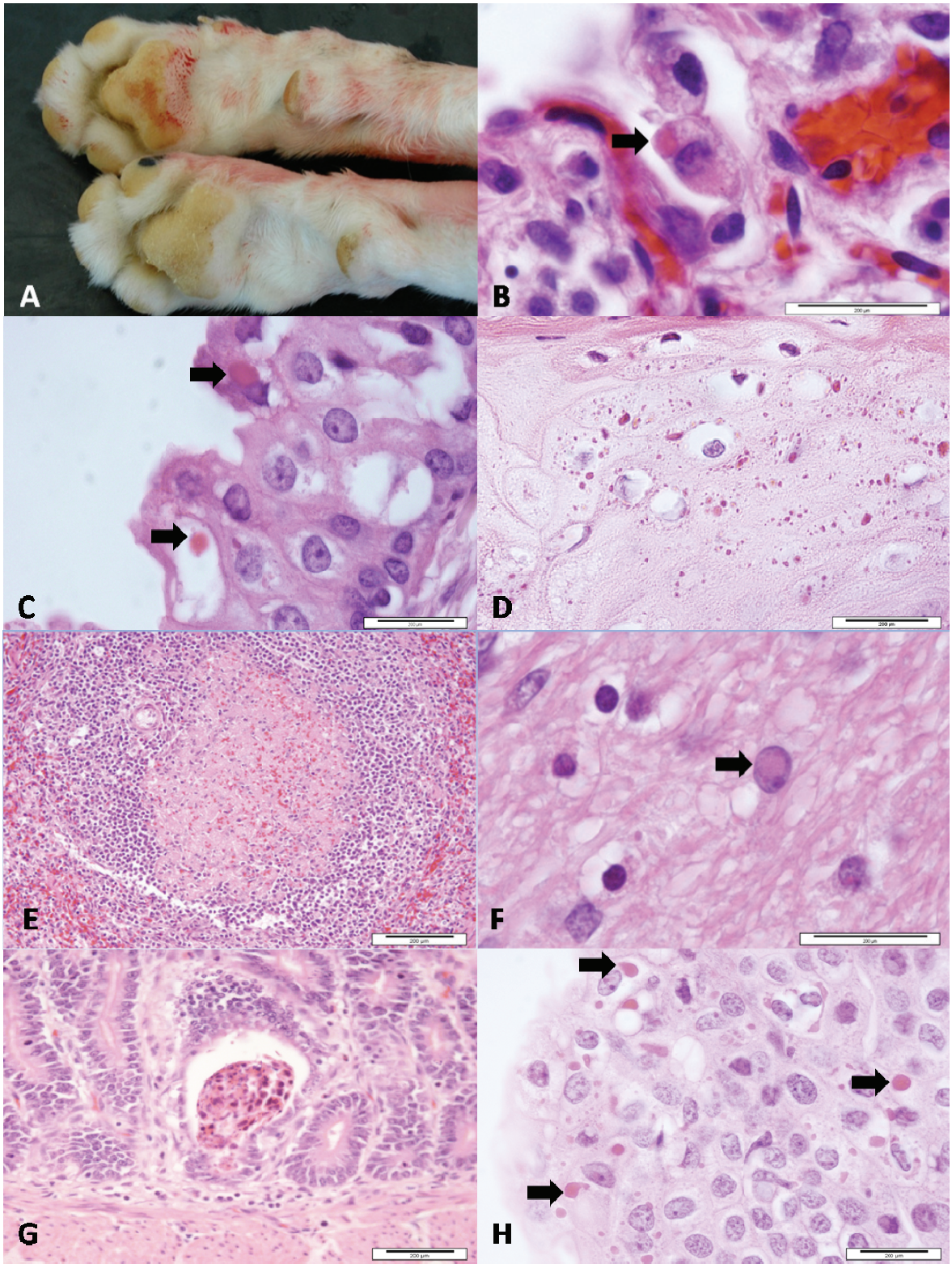

Figure 1 – Dog. Gross and microscopic images of canine distemper virus-induced systemic infection. There is discrete digital hyperkeratosis (A). Observe eosinophilic intracytoplasmic inclusion body (arrow) within epithelial cell of the lung (B) and within degenerated transitional epithelial cells (arrows) of the urinary bladder (C). There is degeneration of keratinocytes of footpad epithelium (D) and necrosis of splenic germinal center (E). Observe distinct eosinophilic intranuclear inclusion body (arrow) within the astrocyte of the cerebellum (F). There is necrosis of intestinal crypt (G) and several intracytoplasmic inclusion bodies (arrows) within the epithelial cells of the renal pelvis (H). B-H, Hematoxylin and eosin; Bar = 200 µm. Bar, B-C, F, H: 20 µm

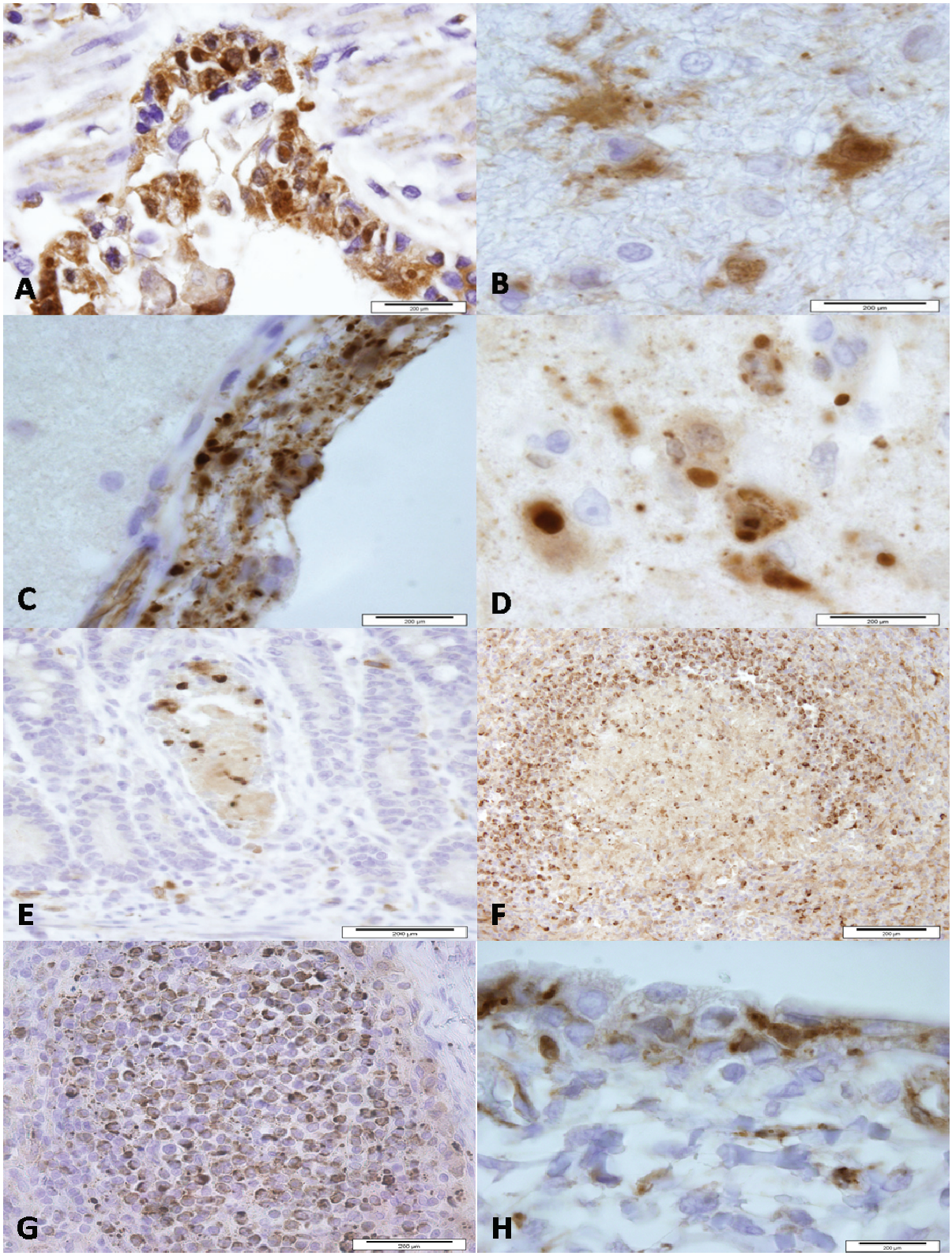

Figure 2 – Dog. Immunohistochemical characterization of canine distemper virus (CDV) antigen. There is intense staining of bronchial epithelial cells (A), astrocytes (B) and epithelial cells of the ependyma of the cerebrum (C), and neurons of the cerebral cortex (D) with CDV antigen. Observe immunopositivity to CDV antigen within intestinal epithelial cells adjacent to an area of cryptal necrosis (E). Lymphocytes of the spleen around necrotic germinal center (F) and of the lymph node (G) contained CDV antigen. There is marked staining of epithelial cells of the renal pelvis by CDV antigen (H). Streptavidin-biotin technique; Bar, A-D,H: 20 µm; E-G, 200 µm.

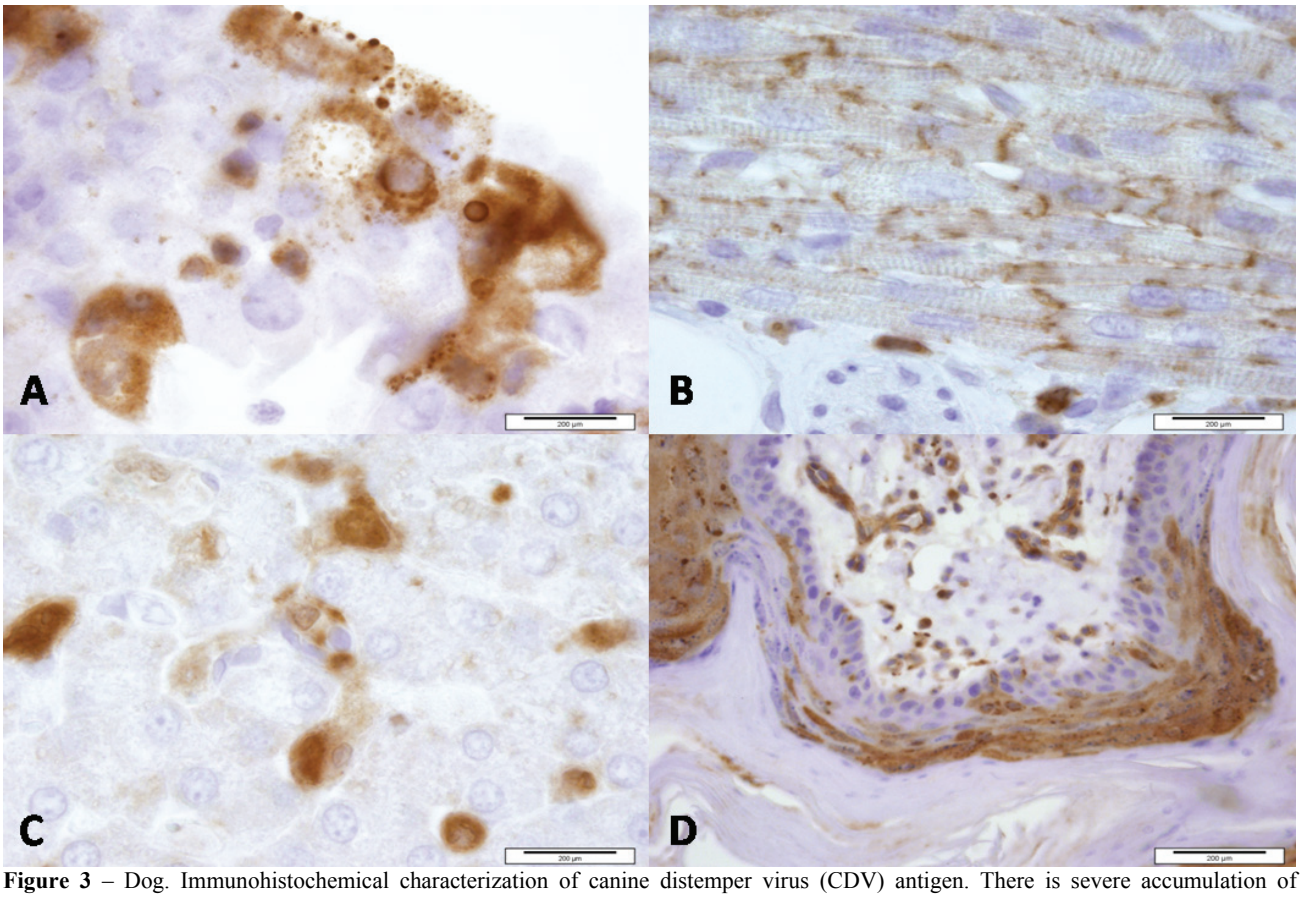

Figure 3 – Dog. Immunohistochemical characterization of canine distemper virus (CDV) antigen. There is severe accumulation of antigen within the dilated transitional epithelial cells of the urinary bladder (A) and cardiac muscle fibers (B). Observe positive reaction to CDV antigen within macrophages and vascular endothelial cells of the liver (C). CDV antigen is positively stained within keratinocytes, fibroblasts, and vascular endothelial cells of the digital epidermis (D). Streptavidin-biotin technique; Bar, A-C, 20 µm; D, 200 µm

immunohistochemistry (IHC). However, the severity of this infection was further extended to include the lymphoid, hepatobiliary, cardiac, and gastrointestinal systems due to multifocal and intense positive immunoreactivity to CDV antigens within lymphocytes and epithelial cells from these tissues. A similar description of severe systemic infection occurred in an experimentally induced case of CDV infection identified by immunofluorescence (2).

These findings indicate that the histologic lesions associated with systemic distemper infection may vary from nonspecific to characteristic in different tissues of the same dog. Therefore, when the lesions of canine distemper are specific, as occurred in the lung, urinary bladder, and renal pelvis, a diagnosis of distemper can easily be made based on typical morphological alterations that are consistent with this disease (4, 6). These alterations are diagnostic for CDV infections in endemic areas of the disease. Further, intranuclear inclusion bodies within astrocytes and neurons without characteristic histological signs of distemper encephalitis (white-matter demyelination and/or perivascular cuffing with an influx of lymphocytic-plasmacytic inflammatory cells) were identified in the brain of this dog, which was confirmed by IHC. In acute clinical disease of young dogs, inflammatory alterations to the brain are minimal probably due to immunodeficiency by physiological immaturity of the immune system of the host or by virus-induced immunosuppresion (6). Consequently, IHC will be necessary to confirm a diagnosis of this infection when lesions are not pronounced or for the characterization of this infection in lymphoid and cutaneous lesions (4, 11), as occurred within the spleen, lymph node, and footpad epithelium of this dog. IHC will also be ideal for the characterization of this disease in countries or geographical locations where the disease is not endemic. Additionally, it was recently indicated that the tonsils might be the ideal tissue to identify inclusion bodies in cases of systemic distemper (12), due to initial viral multiplication in lymphoid tissues (6). Moreover, IHC is superior for the diagnosis of CDV-induced infection compared to the identification of inclusion bodies, but these results are more efficient in acute relative to chronic cases in which the viral antigen may not be expressed (6).

The depletion of lymphocytes, necrosis of splenic germinal centers, and the laboratory finding of leukopenia,

primarily due to lymphopenia, observed in this case are characteristic manifestations of systemic CDV infection in young dogs (4, 6). These lesions are caused by the destruction of T and B cells by the CDV (1, 6). Further, the identification of CDV antigens within the intestines, Kupffer cells of the liver, vascular endothelium of most organs, cardiac muscle fibers, astrocytes and neurons of the brain, and the epithelial cells of the renal pelvis indicates that replication has already occurred within macrophages and lymphocytes (1). With such intense viremia, this dog probably would not have recovered from this systemic CDV-induced infection. This widespread dissemination is related to a cell-associated, plasma-phase hematogenous dissemination in conjunction with a deficient host immune response (6), already compromised by severe lymphoid depletion. Laboratory evaluation also demonstrated anemia and thrombocytopenia in this dog; these hematological abnormalities are not frequently described in CDV-induced infections, are considered non-specific, and might not be directly associated with this disease, but have been related in experimentally infected neonate pups (6).

Footpad hyperkeratosis was discrete in this case, and might not be that significant to be considered as the classical manifestation of digital CDV-induced hyperkeratosis. Additionally, the lesion herein described may probably represent the acute phase of this disease that would have become chronic and more pronounced if the animal was maintained alive for a longer period. Although distinct inclusion bodies were not identified within degenerated keratinocytes by histopathology, these cells contained CDV antigens identified by IHC; CDV antigen was also observed within fibroblasts, vascular endothelium, and the epithelial cells of sebaceous glands and hair follicles of the footpad epidermis. Similar IHC findings were described in naturally occurring (9, 11) and experimentally induced cases of hard pad disease (7, 8). However, the degenerated keratinocytes seen in this case might not be the typical histological feature of dogs with hard pad disease. This lesion did not occur in spontaneous cases (11), but was observed in some dogs experimentally infected with CDV (7, 8). The mild histological alterations of epidermal digital cells observed in experimentally induced and naturally occurring cases of typical hard pad disease have been associated with persistent viral infection and the proliferation of keratinocytes (7, 11).

A unique finding in this dog was the presence of CDV antigens within the lamina propria, vascular endothelium, and epithelial cells of damaged intestinal crypts; diarrhea was not observed clinically, there was no sign of gastrointestinal disease during necropsy, and inclusion bodies were not observed by histopathology. Similar intestinal findings were also observed in an outbreak of distemper in some dogs with CDV-induced nervous lesions and bronchopneumonia but without inclusion bodies in the gastrointestinal and renal systems (13); the authors attributed this occurrence to the difference in viral strain, and the epitheliotropism and cytopathogenic effects of CDV. Additionally, the exact nature of the CDV-induced intestinal lesion is not well established (4). There was discrete cryptal necrosis in this dog whereas epithelial degeneration, necrosis of lymphoid tissue and neutrophilic infiltration were observed in other cases (13).

The finding of CDV antigens within intestinal epithelial cells and the vascular endothelium of the intestine and other tissues of this dog might be related to hematogenous viral dissemination during systemic infection. Hematogenous spread to the central nervous system is considered the principal pathway for viral dissemination (9). This pathway has recently been postulated as an alterative method of CDV entry into the nervous system (15), by inducing lesion to the blood-brain barrier (3), before there are manifestations of the characteristic features associated with distemper-induced encephalitis. Further, the virus probably enters the nervous system of viremic dogs before clinical manifestations of neurologic dysfunction (6). Consequently, hematogenous entry and the late manifestation of neurological lesions might be the key to explain the absence of marked histological CDV-induced brain alterations in this dog when compared to those observed in the lung, urinary bladder, and spleen.

In conclusion, a case of systemic distemper infection is described in a pup. Histology revealed characteristic viral inclusion bodies within epithelial cells of several systems, while immunohistochemistry confirmed the systemic distribution of the disease by positive immunoreactivity to antigens of CDV.

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