NOTE *Clinical Pathology*

Distribution of Inclusion Bodies in Tissues from 100 Dogs Infected with Canine Distemper Virus

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ABSTRACT. One hundred dogs that were positive for canine distemper virus antigen and inclusion bodies in the tonsils were examined for the distribution of inclusion bodies in various tissues. Inclusion bodies were found in the lungs (70 dogs), brains (20 dogs), urinary bladders (73 dogs), stomachs (78 dogs), spleens (77 dogs), and lymph nodes (81 dogs) of the dogs. Based on these results, the tonsils may be the most suitable tissue for detection of inclusion bodies in canine distemper.

KEY WORDS: canine distemper virus, inclusion body, tissue distribution.

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Canine distemper virus (CDV), which is included in genus Morbillivirus of family Paramyxoviridae, has been demonstrated in various cells of different tissues and organs [1]. This pantropic and highly contagious virus causes systemic and often fatal disease in domestic dogs. CDV vaccines are available for use in dogs. However, sporadic cases of CDV infection sometimes break out even in domestic dog populations in which broad vaccination coverage is maintained. Therefore, accurate diagnosis of this disease at an early stage is required to quarantine any infected dogs and to prevent the disease from spreading. Inclusion bodies in the brain, lungs and urinary bladder are reliable indicators for post mortem diagnosis of CDV infection [1]. Viral antigen can also be demonstrated and confirmed in these organs by immunohistochemical techniques [1, 2]. Some reports have described the virus distribution in limited organs, such as the lung, brain, urinary bladder, gastrointestinal tract, foot pads, and lymphoid organs [1–5]. To our knowledge, however, the incidence of CDV distribution in organs has not been reported. In this study, 100 canine necropsy cases with CDV infection were histologically examined for inclusion bodies in order to effectively clarify the most suitable organ for diagnosis of CDV infection.

One hundred dogs that were presented to Rakuno Gakuen University for necropsy between 1995 and 2002 were immunohistochemically confirmed to have CDV antigen in the reticuloendothelial cells of the tonsils. Immunohistochemical staining was performed for CDV using the avidin-biotin-peroxidase complex (ABC) procedure (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA, U.S.A.). The primary antibody was rabbit polyclonal for CDV (Nisseiken, Tokyo, Japan). The dogs were 2- to 4- months old and of various breeds. The clinical course was from a few weeks to 2 months. These dogs were suspected to have CDV infection based on clinical symptoms. They had not been vaccinated and had a range of various clinical signs, including nasal discharge, cough, pneumonia, diarrhea, conjunctivitis, scaling, crusting, hard pads, and neurological symptoms. They died or were euthanized due to their poor prognoses. Tissue samples were collected from lungs, brains, urinary bladders, stomachs, spleens, various lymph nodes, and tonsils. The tissues were processed by conventional methods, embedded in paraffin wax, sectioned at 4 μm, and stained with hematoxylin and eosin (HE) for histopathological examination. The main necropsy findings were pneumonia. Pneumonic lungs were edematous. Dark red, sharply demarcated patches of consolidation were seen in the lobes. Spleens, thymuses and lymph nodes were atrophic. Histopathology also revealed inclusion bodies in the tonsils of all cases. Intracytoplasmic and intranuclear inclusion bodies were found in multiple organs from these cases, including the lungs, brains, urinary bladders, stomachs, spleens, and lymph nodes. Specifically, CDV inclusion bodies were found in the lungs (70 dogs), brains (20 dogs), urinary bladders (73 dogs), stomachs (78 dogs), spleens (77 dogs), and lymph nodes (81 dogs) of the 100 dogs (Fig. 1).

Significant changes were detected in the lymphoid tissues. The tonsils, spleens and lymph nodes were remarkably atrophic with depletion of lymphocytes and necrosis in follicles. Intracytoplasmic acidophilic inclusion bodies were occasionally seen in reticuloendothelial cells and lymphocytes (Fig. 2). CDV antigen was observed in reticuloendothelial cells by Immunohistochemistry (Fig. 3).

Post mortem examination for CDV is one of the most sensitive methods to confirm infection. In a previous study, stomach mucosa, conjunctiva, and urinary mucosa were reported to be the most useful organs for detection of CDV antigen, since CDV is epitheliotropic in nature [1]. However, in this study, the detection rates of inclusion bodies in different organs were extremely high for systemic lymphatic organs, including the tonsils, spleen, and lymph nodes. CDV replicates in lymphoid tissues and spreads to

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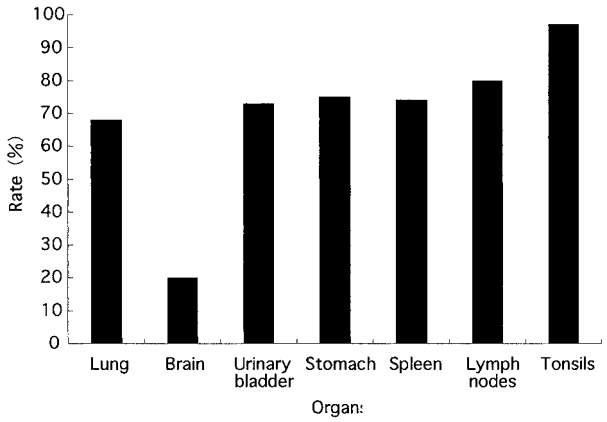

Fig. 1. Distribution rate of inclusion bodies. CDV intracytoplasmic and intranuclear inclusion bodies were found in 70% of the lungs, 20% of the brains, 73% of the urinary bladders, 78% of the stomachs, 77% of the spleens, 81% of the lymph nodes, and 100% of the tonsils.

other tissues via infected lymphocytes. Since the natural route of CDV infection is inhalation [1, 4], virus replication would occur first in the cells of the lymphatic tissues of the respiratory tract. Therefore, CDV can be detected in tonsils at an early stage [1, 8]. CDV infection in dogs induces marked depletion of lymphocytes in both T- and B-cell dependent areas of the lymphoid tissues in the early stages of infection, as is the case for other morbillivirus infections, such as measles and rinderpest [3, 6, 7]. In fact, previous studies support the above process of infection [1, 4], and abundant CDV antigen is most frequently detected from the early to late stages of infection in lymphoid organs, especially the tonsils [8].

Histological examination of biopsy samples from the tonsils could be sensitive and specific for diagnosis of CDV infection, since the present study revealed that CDV inclusion bodies are highly detected in the tonsils. Therefore, histological and immunohistochemical examination of biopsy samples from the tonsils could become the preferred method for diagnosis of CDV infection, since the tonsils are lymphatic organs of the upper respiratory tract that are fairly easy to biopsy.

However, in our experience with 300 necropsy cases clinically diagnosed with CDV, no CDV inclusion bodies were detect in the tonsils of 3 of the cases; CDV inclusion bodies were detected in the cerebrum only (data was not shown). These 3 cases were considered to have cleared the CDV from the lymphoid tissues and other epithelial cells but not the neural tissue in the chronic stage. In cases of chronic CDV infection or those with neurological signs only without other typical symptoms, a more sophisticated procedure is required to diagnose CDV infection.

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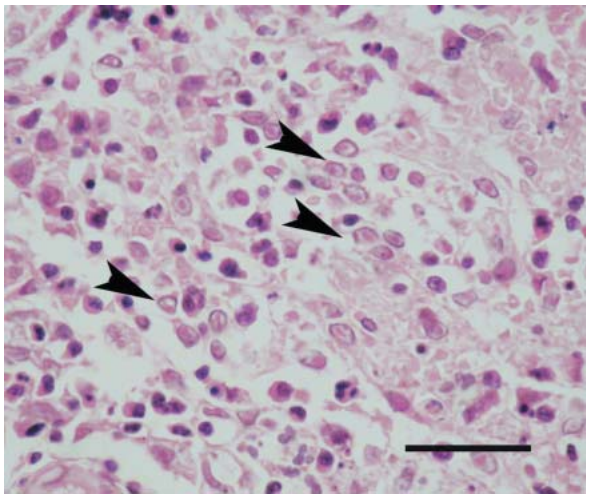

Fig. 2. Spleen: The number of the lymphocytes is severely decreased, and many intranuclear acidophilic inclusion bodies can be seen in the reticuloendothelial cells (arrow). Hematoxylin and eosin staining. Bar=50 μm.

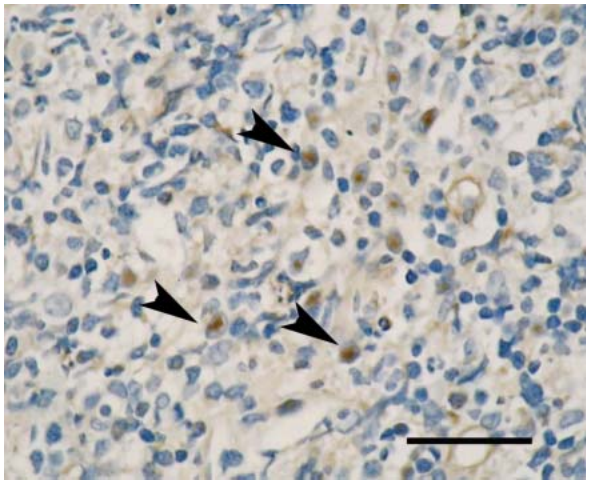

Fig. 3. Tonsils: The reticuloendothelial cells are positive for anti-CDV antibody (arrow). Streptavidin-biotin-peroxidase method with Mayer's hematoxylin counterstaining. Bar=50 μm.

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