

Dual Infection with Canine Distemper Virus and Infectious Canine Hepatitis Virus (Canine Adenovirus Type 1) in a Dog

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ABSTRACT. A 72-day-old, female, Golden Retriever dog showed anorexia, coughing, nasal discharge, diarrhea and hematochezia, and died on the 15th clinical day. Pathological examination revealed dual infection with canine distemper virus (CDV) and canine adenovirus (CAV). CAV inclusion bodies occurred only in the liver, and biliary and respiratory system, whereas CDV inclusions were demonstrated in the visceral organs systematically. The CAV inclusions were associated with multifocal hepatocellular necrosis and edematous swelling of the wall of the gall bladder, suggesting infectious canine hepatitis virus (canine adenovirus type 1) infection.—**KEY WORDS:** canine distemper virus, dual infection, infectious canine hepatitis virus (canine adenovirus type 1).

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Both canine distemper virus (CDV) and canine adenovirus are known to cause respiratory disease [7, 8, 13, 16]. There are two types of canine adenovirus (CAV), canine adenovirus type 1 or infectious canine hepatitis virus (ICHV) and canine adenovirus type 2 or Toronto A26/61 virus [12]. Both these types have been isolated from throat swabs of dogs with respiratory symptoms [1]. Experimentally, an aerosol inoculation of ICHV has never induced any systemic lesions of infectious canine hepatitis, except for only the presence of intranuclear inclusion bodies in hepatocytes without hepatic necrosis [14, 15]. It had been assumed that a simultaneous infection with both CDV and CAV was more common in the field, although it has been reported rarely in literature [11]. There are only four reports on spontaneous dual infection with CDV and CAV [2, 3, 10, 11]. In these reports, however, hepatic necrosis associated with the presence of adenovirus particles has not been described.

Prydie *et al.* [9] demonstrated that CDV and ICHV could coexist in the same cell culture and even in the same cell. However, there is only one case report which demonstrated that both CDV and CAV replicated in one and the same cell [2]. This note reports on a dual infection of CDV and CAV in a dog with both types of viruses replicating in one and the same cell in the lung, and hepatic necrosis associated with adenovirus inclusion bodies.

A 72-day-old, female, Golden Retriever dog died on a 15-day clinical course with anorexia, coughing, nasal discharge, diarrhea and hematochezia. A blood examination carried out on the 8th day of the course revealed a moderate increase in total white blood cell count (25,500/mm³), anemia (hematocrit; 20%) and hypoproteinemia (total protein; 3.4 mg/dl). After necropsy performed by a veterinary clinician, all the visceral organs and the brain from the dog were fixed in formalin and submitted to our Department for pathological examination.

Prominent pulmonary edema with grayish spongy-like appearance, marked liver opacity with meat-like and anemic appearance, mucosal petechial hemorrhage in the urinary bladder and dilatation of right cardiac ventricle were observed in formalin-fixed organs.

Histopathological examination was carried out routinely after refixation in 10% neutral buffered formalin. As a

result, a dual infection with CDV and CAV was demonstrated. The CAV infection involved only the liver, and biliary and respiratory system, whereas CDV infection occurred in the visceral organs systematically, including the respiratory, biliary, pancreatic, urinary, alimentary, and genital systems and the spleen.

In the lung, broncho-interstitial pneumonia was the most prominent finding with marked congestive edema. Focal fibrinous exudation was also found in the alveoli with a moderate proliferation of alveolar type 2 cells. The bronchial and bronchiolar epithelial cells were hyperplastic, some of which were necrotic and degenerative. In these lesions, there were both basophilic, amorphous, Cowdry type A intranuclear inclusion bodies, and eosinophilic intranuclear and intracytoplasmic inclusion bodies in the epithelial cells of the bronchi, bronchioli, alveoli and bronchial glands, as well as in alveolar macrophages. Ultrastructurally, the former type of the inclusions were usually filled with large numbers of viral particles, approximately 70 to 80 nm in diameter. The particles showed a crystalline arrangement with or without an electron dense core. These morphological characteristics of the viral particles resembled those of adenovirus. Groups of the viral particles were also seen in the cytoplasm of the destructed cells and extracellular spaces in the necrotic foci. The latter type of the inclusions consisted of electron dense amorphous mass or filamentous clumps. These particles often had an irregular striation representing transverse sectioned nucleocapsid filaments, 15 to 20 nm in diameter. These morphological characteristics of the viral particles were similar to those of paramyxoviruses (CDV). In a limited number of cells involved in the lung, both the adenovirus and paramyxovirus inclusions were present within the nucleus and cytoplasm of the same cell, respectively (Fig. 1).

In the trachea, there was epithelial cell necrosis and/or degeneration accompanied with both types of inclusions and mild inflammatory cell infiltration. Similar findings were also seen in the epithelial cells of the tracheal glands.

The liver had multifocal hepatocellular necrosis, especially in the periportal regions. These areas contained fibrin, cell debris and a small number of inflammatory cells. Adjacent to these foci, intranuclear basophilic to amphophilic, Cowdry type A inclusions were seen in the

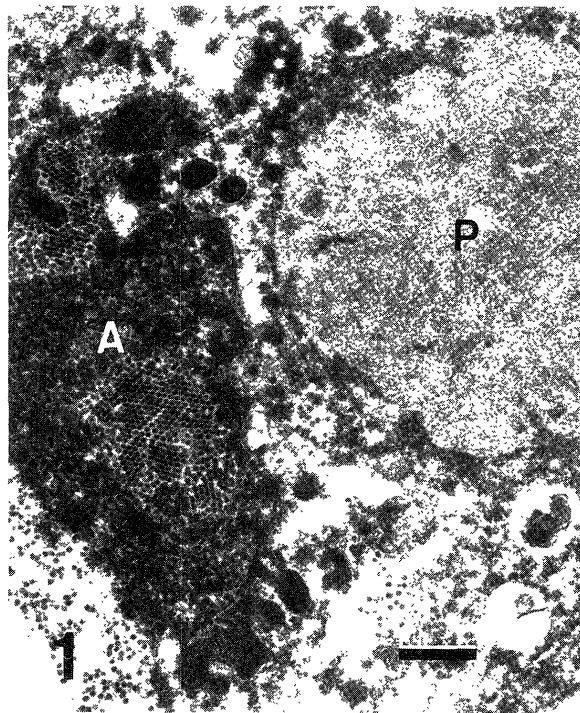


Fig. 1. An alveolar macrophage having intranuclear and intracytoplasmic inclusion bodies induced by adenovirus (A) and paramyxovirus (P), respectively. The adenovirus inclusion consists of viral particles showing crystalline arrangement, and the paramyxovirus inclusion is composed of nucleocapsids. Adenovirus particles are also seen in the degenerated cytoplasm, Bar = 1 μ m.

hepatocytes and Kupffer's cells (Fig. 2). Ultrastructurally, these inclusions consisted of the same adenovirus particles as those found in the lung. Similar particles were also present in the severely destroyed cytoplasm of the hepatocytes and Kupffer's cells, and extracellular spaces within fibrin clumps. At the periphery of the necrotic foci the hepatocytes showed marked vacuolar degeneration. Fibrin thrombi were also seen in dilated sinusoids. Portal tracts were often edematous. The epithelial cells of interlobular bile ducts also showed necrosis and/or degeneration with both types of inclusions similar to those in the lung. Although the gall bladder showed severe postmortem change, it had mild to moderate edematous thickening of the wall with dilatation of lymph vessels and epithelial hyperplasia. In addition, shadows of both types of inclusions were detected in the epithelial cells.

Additional microscopic findings in this animal included systemic lymphocytic depletion and necrosis in the lymphoid organs, and subacute suppurative enteritis due to coccidiosis.

Lesions of the respiratory system in the present case coincided with those of the previous reports on both CDV and CAV infection [2, 3 10, 11]. Gillespie *et al.* [5] demonstrated that experimental inoculation of both CDV and ICHV to puppies induced dual infection with increased mortality. However, no report has described dual infection of CDV and ICHV accompanied with hepatic



Fig. 2. Periportal focal necrosis of the liver associated with intranuclear inclusion bodies in hepatocytes and Kupffer's cells (arrows), and fibrin exudation. Phosphotungstic acid hematoxylin stain. $\times 270$.

and biliary lesions. The lesions of the liver and gall bladder in the present case suggest that the adenovirus belongs to ICHV, canine adenovirus type 1. It has been said that dogs immune to parenteral challenge with ICHV were still susceptible to respiratory disease via aerosolized viral particles [6]. Vaccination against CAV can not prevent infection by this virus, but it may reduce the severity of clinical signs [4]. Thus, in aerosol ICHV infection associated with CDV infection, the latter induces immunosuppression and may affect the severity of ICHV infection in puppies.

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