



## Case Report

# Canine adenovirus type-2 and canine distemper virus pulmonary co-infection in two Chow-Chow puppies with *Candida sp* esophagitis

Adriane L. Gabriel<sup>1</sup>, Eduardo K. Masuda<sup>1</sup>, Adriano T. Ramos<sup>1</sup>, Maria A. Inkelmann<sup>1</sup>,  
Ellen Azambuja<sup>2</sup>, Dominguita L. Graça<sup>3</sup>

<sup>1</sup> Laboratory of Veterinary Pathology, Federal University of Santa Maria (UFSM), RS, Brazil.; <sup>2</sup>Mundo Animal Veterinary Clinic, Santa Maria, RS, Brazil. <sup>3</sup> Department of Pathology, Federal University of Santa Maria (UFSM), RS, Brazil.

\*Corresponding author: Dominguita Lühers Graça. Rua Engº Luiz Bollick, 330. Bairro Nº Sra. de Lourdes 97050-210, Santa Maria, RS. Email: [dlgraca@gmail.com](mailto:dlgraca@gmail.com).

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## Abstract

Two three-month-old puppies from the same litter were submitted to necropsy after a clinical history of purulent ocular discharge, diarrhoea and coughing. Grossly, the lungs were filled with fluid, firm and diffusely mottled with red and yellow areas. A clumped white-yellowish material with longitudinal stripes was loosely adhered to the esophagus. The histological analysis of the lungs revealed a suppurative and histiocytic broncho-interstitial pneumonia with fibrin, hemorrhage and myriads of eosinophilic intracytoplasmic and basophilic intranuclear inclusion bodies. The diagnosis was consistent with co-infection by canine adenovirus type-2 and canine distemper virus. Both dogs also had severe proliferative *Candida sp.* esophagitis. Reports of two concomitant viral diseases and mycotic infection are relatively rare and suggest intrinsic and environmental immunosuppressive factors.

**Key Words:** canine distemper, canine adenovirus type 2, *Candida sp.*, diseases of dogs, immunohistochemistry

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## Introduction

Viral respiratory diseases in dogs can be associated with canine distemper virus (CDV) and/or with canine adenovirus type 2 (CAV-2). They both represent a severe health problem, specially in commercial kennels and veterinary hospitals (4,6).

Canine distemper is a highly contagious multisystemic disease caused by *Morbillivirus* of the Paramyxoviridae family, which is mainly transmitted by aerosols and secretions from the respiratory tract (4,17,18). It replicates within tonsils and bronchial lymph nodes. The infection persists on lymphoid tissues if the antibody levels are low, disseminating the virus to the epithelial cells of

the alimentary, respiratory and urogenital tracts, skin and brain (14), leading to respiratory, gastrointestinal and neurologic signs (6). Lesions of the respiratory tract are common and are often characterized by moderate to severe bronchointerstitial pneumonia (5). Histologically, the bronchioles are expanded by a suppurative exudate, with necrosis of bronchial/bronchiolar epithelium. Edema, fibrin, mononuclear and necrotic cells are frequently observed inside the alveoli. Characteristic findings also include: syncytial giant cell formation, acidophilic intracytoplasmic inclusion bodies in bronchial/bronchiolar epithelial cells and type II pneumocytes hyperplasia (5). Canine distemper inclusion bodies can range from 1 to 5 µm and can also be found in other organs, such as the

gastric mucosa, transitional epithelium of the urinary bladder and the brain (5). Viral immunosuppressive ability can lead to concomitant and opportunistic infections (15,18). Secondary bronchopneumonia can be present, especially in subacute to chronic cases (5).

CAV-2 belongs to the genus *Mastadenovirus*, of the Adenoviridae family. It is one of the agents involved in canine infectious laryngotracheitis (AKA "kennel cough") (13). This group of diseases is characterized by fever, ocular and nasal discharges, coughing and emaciation (16). Viral infection occurs through the oronasal route, replicating mainly in the upper respiratory tract. However, non-ciliated epithelium of bronchi and bronchioles and type II pneumocytes can also be affected (3). Pulmonary lesions are characterized by bronchointerstitial pneumonia, with epithelial necrosis and basophilic intranuclear inclusion bodies in the bronchiolar and alveolar epithelium (1). CAV-2 infection is generally related to mild clinical signs. Nevertheless, association with others infectious agents can induce more severe lesions (10). Simultaneous infections with CAV-2 and CDV have already been described (1,2,6).

*Candida* sp. is a dimorphic fungus of the Cryptococcaceae family which is commonly found in the alimentary, upper respiratory and genital tracts of humans and animals (8). In normal conditions, this microorganism does not cause disease. However, *Candida* sp. proliferates when there is instability of microbiota, leading to an opportunistic fungal infection (11). Immunosuppressive factors, such as CDV infection or the use of antibiotics can collaborate to the development of local or systemic mycotic diseases (9,19). Until now, there are no reports associating *Candida* sp. and CAV-2 infection.

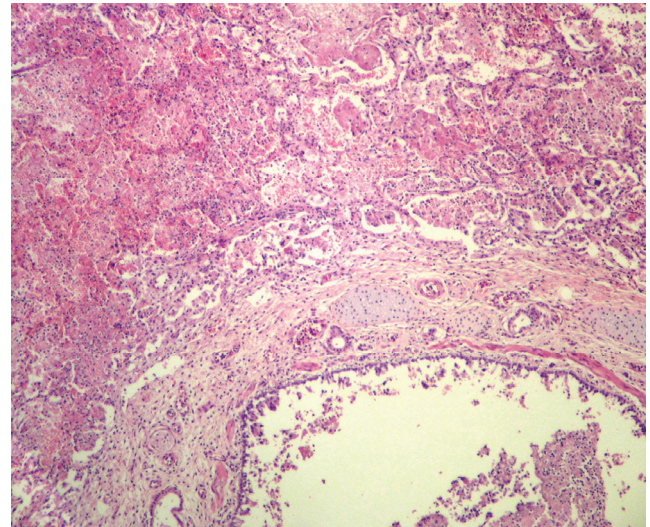
The purpose of this report is to describe the clinical and pathological findings of a rare triple infection for CDV, CAV-2 and *Candida* sp. in two Chow puppies from the same litter.

## Case Report

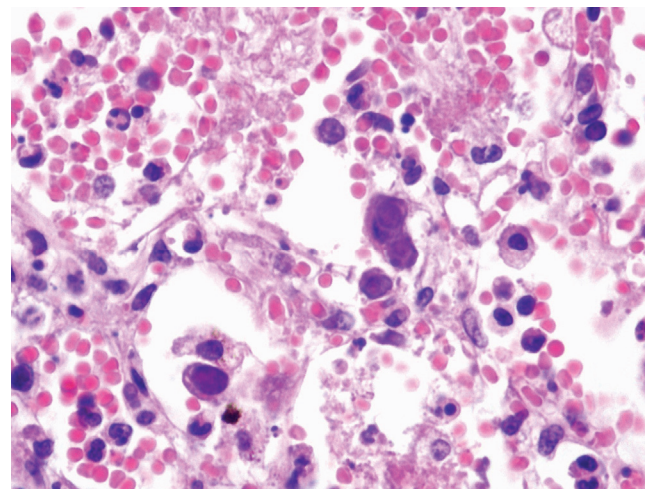
Two three-month-old Chow dogs from the same litter, one male (dog no.1) and one female (dog no.2) were presented to a veterinary clinic with clinical signs of coughing and diarrhoea. The two dogs died despite the treatment with ampicillin and amoxicillin and they were sent to the Veterinary Pathology Laboratory (LPV) at the Universidade Federal de Santa Maria for necropsy. At gross examination, the dogs were in good condition despite the paleness of all mucous membranes. Dog no.2 had also a bilateral purulent ocular discharge. The lungs were enlarged, firm, yellow to dark-red diffusely mottled. Longitudinal yellow-whitish stripes were loosely adhered to the esophagic mucosa. No changes were seen in other organs.

Microscopically, the lungs of both dogs had a severe fibrinous and suppurative bronchopneumonia characterized by an extensive neutrophilic inflammatory

infiltrate in bronchioles and alveoli (Figure 1). Fibrin, degenerated neutrophils, foamy macrophages, hemorrhage and desquamated epithelial cells were also observed inside the alveoli (Figure 2).



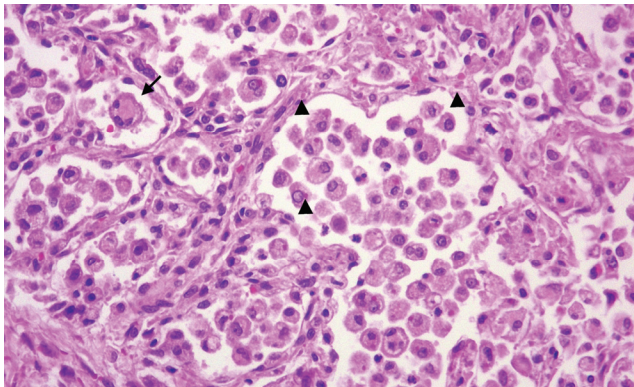
**Figure 1.** Area of pneumonia around a bronchus where hemorrhage, inflammatory filled alveolar spaces and fibrin are observed (HE, Obj. 10X).



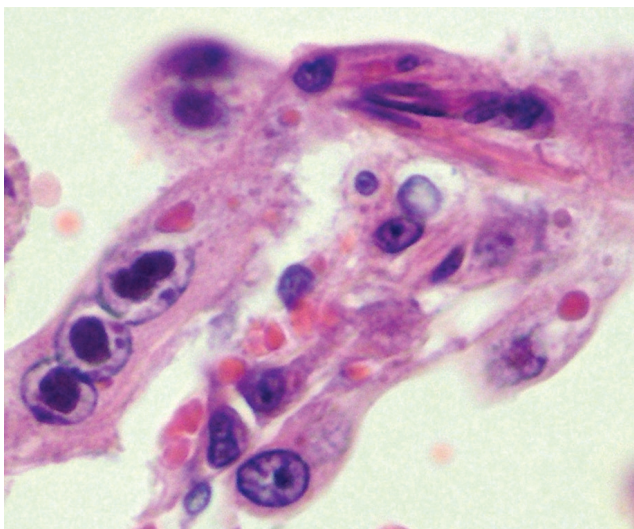
**Figure 2.** Area of necrosis and hemorrhage where large CAV-2 basophilic intranuclear inclusion bodies are seen within histiocytes (HE, Obj. 20X).

Multiple foci of severe bronchiolar and alveolar necrosis were accompanied by syncytial cells (Figure 3). Acidophilic intracytoplasmic inclusion bodies were observed in some bronchiolar cells (Figure 3). Similar inclusion bodies were also found in the urinary bladder, esophagus and gastric epithelia. Large basophilic intranuclear inclusion bodies filled the nucleus of many scattered alveolar cells, type II pneumocytes, epithelial bronchiolar cells and histiocytes, in some areas concomitant with acidophilic intracytoplasmic inclusion bodies (Figure 4). Multiple areas of epithelial hyperplasia of the esophagic mucosa were observed accompanied by severe hyperkeratosis and desquamation of cells. Fibrin

filaments, basophilic coccoid bacterial aggregates and myriads of negative-stained leveduriform and pseudo-hyphae structures were also observed. Thin-walled ovoid leveduriform structures had a diameter of 3 to 6  $\mu\text{m}$ . Pseudo-hyphae were cylindrical and branched, and measured approximately 10 $\mu\text{m}$  width to 150 $\mu\text{m}$  length, with thin lightly basophilic walls. These fungal elements were morphologically consistent with *Candida* sp. and were positively stained with PAS (Periodic Acid Schiff reaction) (Figure 5).



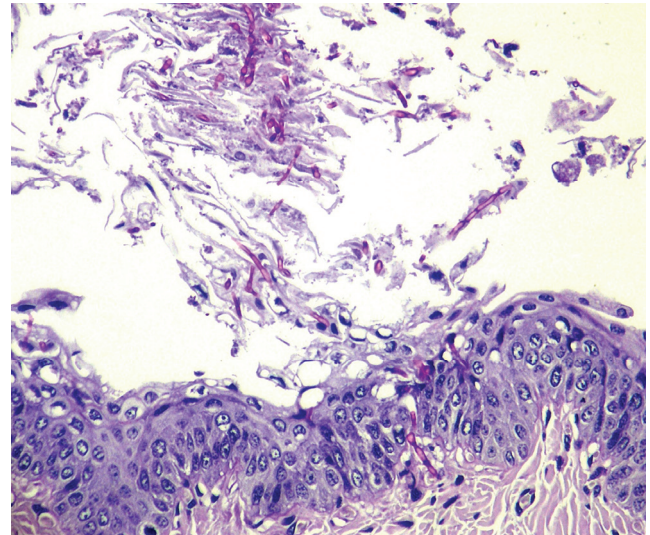
**Figure 3.** Alveolar spaces are filled with foamy macrophages which exhibit CDV intracytoplasmic eosinophilic inclusion bodies (arrowheads) also seen in alveolar epithelial cells. Syncytia are also found (arrow). HE, Obj. 20X.



**Figure 4.** In some areas both types of inclusion bodies (CDV and CAD-2) were detected (HE, Obj 40X).

Three micrometer histological sections from lung and urinary bladder were submitted to immunohistochemistry using the streptavidin-biotin-peroxidase method. Briefly, after the antigen retrieval in a boiler for 3 minutes, the slides were incubated with anti-CDV antibody (1:400, VRMD Pullman, WA, USA) overnight at 4°C. The sections were then incubated with a secondary byotinitated antibody and LSAB complex. Immunoreactivity was visualized using Permanent Red (DakoCytomation, CA, USA). Sections were

counterstained with Harris's haematoxylin, mounted with permanent mounting medium and examined by light microscopy. In this case, the lung and urinary bladder were negatively immunostained.



**Figure 5.** Hyperplastic esophagic mucosa where PAS-positive *Candida* sp pseudo-hyphae are observed within the epithelium and in desquamated tissue within the lumen of the organ (PAS, Obj. 20X).

## Discussion

Pulmonary simultaneous infection with CDV and CAV-2, associated with mycotic esophagitis by *Candida* sp. in dogs has not yet been reported. Although the absence of immunostaining for CDV, the lesions and the presence of acidophilic intracytoplasmic inclusion bodies in epithelial cells of lungs, esophagus, stomach and urinary bladder confirm the diagnosis of canine distemper (1,18). Many factors might be used to explain the lack of immunostaining in the present case. In formalin-fixed paraffin-embedded tissues, an improper tissue fixation (delayed, over or underfixation) may influence the final result. For some antibodies, a delay in fixation may alter antigen structure. Processing factors can also be involved. Heat-sensitive epitopes can be lost by embedding in paraffin that is too hot (7). Ghadially (1988) offers an alternative explanation for the lack of immunoreactivity for CDV on tissue sections. Although the presence of inclusion bodies is a striking characteristic of CDV infection, it is believed that many of these corpuscles are actually distemper-like corpuscles not composed by viral protein. Instead, they are made of an accumulation of the cytoskeleton components due to the cytolytic ability of the virus (19).

Basophilic intranuclear inclusion bodies observed in the present case are also diagnostic for adenoviral infection (1). Both CDV and CAV-2 are highly contagious infections and the transmission occurs mainly after the direct contact with infected dogs (13). Highly populated environments, such as kennels and veterinary clinics, predispose to the diseases, what possibly contributed for

the development of the pulmonary lesions in the present report. Despite the use of effective vaccines, canine distemper remains endemic in most parts of the world, affecting dogs with 3 to 6 months of age when maternal passive immunity decreases (14). On the contrary, highly effective vaccines for CAV-2 have reduced the disease within the canine population. However, the infection still have been diagnosed, especially in immunosuppressed dogs (1). In the present report, vaccination status of the bitch and the puppies were not available.

Concurrent infections with CDV and CAV-2 are uncommon but have been occasionally reported (1). The immunosuppressive effect of CDV have been implicated in the pathogenesis of the co-infection with CAV-2, contributing to the viral replication and therefore, to the severity of pulmonary lesions (1,11). Canine Adenovirus Type 1 (CAV-1) and CAV-2 co-infection have also been described, leading to simultaneous bronchointerstitial pneumonia and hepatocellular necrosis (4). In those cases, polymerase chain reaction (PCR) and in-situ hybridization (ISH) are useful tools to distinguish both adenoviruses (3). Although these techniques were not applied in this case, the absence of hepatic lesions and basophilic intranuclear inclusion bodies in hepatocytes excludes CAV-1 infection (1).

Secondary bacterial and/or protozoan co-infection, especially with *Toxoplasma gondii* and *Neospora caninum*, are also related with the immunosuppressive effect of CDV (12), but they were not observed in the present case. Nevertheless, esophagic lesions were compatible with a mycotic infection. Linear yellow-whitish stripes loosely adhered to the esophagic mucosa are called pseudomembranes and are characteristic of candidiasis (9,11). PAS-positive leveduriform and pseudo-hyphae structures are morphologically compatible with *Candida* sp. and are determinants in the establishment of the diagnosis (15). Despite the low clinical significance of the esophagitis, it demonstrates the diminished immunological status of both dogs. A combined pathogenesis (CDV immunosuppression and antimicrobial therapy) was used to explain mycotic proliferative lesions in the present case (9,13). Although *Candida albicans* is frequently isolated from normal and diseased genital and alimentary tracts, other species can also be involved (13,16). As they are morphologically indistinguishable, fungal culture, molecular or immunological assays are necessary to establish a definitive identification (11).

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