Detection of coronavirus in cases of tracheobronchitis in dogs: A retrospective study from 1971 to 2003

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Canine infectious tracheobronchitis (CITB) is a multifactorial disease that commonly occurs when dogs are brought together for boarding, shows, or field trials (1). Generally, the disease is an irritating, but self-limiting, condition; however, it can progress to bronchopneumonia in some cases, especially in individuals that may be immunosuppressed for a variety of reasons (1). Aside from the recognized environmental and physiological, stress-related, cofactors, several infectious agents, including *Canine adenovirus* type 2 (CAV-2) (2), *Canine parainfluenzavirus* (3), and *Bordetella bronchiseptica* (4), have historically been implicated in a causal role. Other less-frequently recognized agents that have been causally associated with CITB include *Canine herpesvirus* (5) and *Mycoplasma* spp. (6). Most recently, a group 2 *Canine coronavirus* was implicated as an important and prevalent infectious cofactor in many cases of respiratory disease in dogs in humane shelters in the United Kingdom (7). This virus is closely related genetically and antigenically to *Bovine coronavirus* and *Human* (respiratory) *coronavirus* strain OC43 and is distinct from enteric *Canine coronavirus* (7). The prevalence of respiratory *Canine coronavirus* in other parts of the world is currently unknown. The objective of this study was to determine if *Canine coronavirus* could be implicated as an etiologic agent in cases of canine respiratory disease. A retrospective immunohistochemical study of archival case material was used as an initial approach to addressing this issue.

One hundred and twenty-six cases of canine respiratory disease, comprising cases from 1971 to 2003, were selected from postmortem material in the pathology files at the Western College of Veterinary Medicine. The selection criteria were final histological morphological diagnoses of tracheitis, bronchitis or bronchiolitis, or both.

Paraffin blocks of formalin-fixed archival tissue were cut and stained immunohistochemically, using previously described techniques (8), with a 1/25 000 dilution of a lapine anti-bovine coronavirus (BCV) antiserum (a gift from Dr. G. Cox, Veterinary Infectious Disease Organization, University of Saskatchewan, Saskatoon). This antiserum was used due to 1) the unavailabily of antibodies against this *Canine coronavirus;* 2) the close genetic and antigenic relationship between BCV and group 2 *Canine coronaviruses,* including the spike genes/proteins (7); and 3) the published precedent for the use of BCV antigen in an enzyme-linked immunosorbent assay (ELISA) to measure antibodies against group 2 *Canine coronaviruses* (7). Slides from a case of bovine coronaviral enteritis were stained simultaneously with the lapine antiserum to *Bovine coronavirus* and an irrelevant lapine serum to serve as positive and negative controls, respectively.

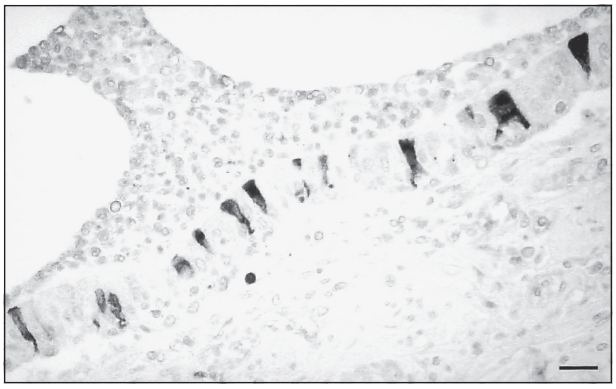

Figure 1. Numerous coronaviral antigen-positive bronchial epithelial cells in a case of suppurative bronchitis in a canine distemper virus-negative puppy that was euthanized due to respiratory disease. Avidin-biotin complex immunhistochemical stain. Bar = 50 μm

Coronaviral infection was demonstrated in 2 cases of bronchitis/bronchiolitis from dogs necropsied in January and February of 1996. Both dogs originated from the local humane shelter. One of the dogs was euthanized during an outbreak of canine distemper and its lung was also positive for *Canine distemper* virus antigen. In both cases, coronaviral antigens were present multifocally in columnar epithelial cells in the bronchi or large bronchioles (Figure 1). In both cases, there was an associated suppurative bronchiolitis.

The results of this retrospective study provide further evidence that *Canine coronavirus* may be etiologically associated with airway disease in dogs. Furthermore, they demonstrated that respiratory infection of dogs with a group 2 coronavirus is not restricted to the United Kingdom (7). However, the results of this and the previous study do not preclude the possibility that this is an emergent virus, since there are currently no data that establish

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infection of dogs with the group 2 coronavirus prior to the late 1990s.

The reliance on postmortem material for this investigation likely underestimates the prevalence of infection with *Canine coronavirus* in cases of CITB for at least 3 reasons: 1) Similar to infection of humans with the closely related *Coronavirus,* OC43, an etiologic agent in the "common cold," most cases of CITB would be self-limiting and not lead to the death of the infected animal. 2) Due to the likely relatively short course of respiratory coronavirus infections, the airways of dogs that die of subacute respiratory disease with bacterial pneumonia (as in the cases examined herein) are unlikely to contain coronaviral antigens at the time of postmortem examination. 3) The latter possibility is supported by the low prevalence of detection of antigens of respiratory viruses in feedlot cattle that die of bacterial bronchopneumonia (D. Haines, unpublished data), despite incriminating evidence of the cattle's seroconversion to viruses, including *Bovine coronavirus* (9). There may have also been some unavoidable sampling bias, since most tissue blocks examined did not contain trachea, which may be the primary site of coronaviral replication and associated lesions.

The results of this study further support the suggestion that coronavirus should be considered in the differential diagnosis of respiratory disease in dogs (7). Moreover, this agent should be considered in cases of apparent "vaccine failure" in outbreaks of respiratory disease in dogs; that is, in situations where dogs that have been fully vaccinated develop respiratory disease after kenneling or other exposure to infected dogs. Since there are currently no commercial vaccines for dogs that contain the group 2 *Canine coronavirus,* available vaccines would not provide clinical protection to this virus. Based on the significant antigenic dissimilarity between the enteric *Canine coronavirus* and the respiratory *Canine coronavirus* (only about 20% identity of amino acids in the immunologically important spike protein) (7), it is highly unlikely that dogs vaccinated with the enteric virus would be protected from disease associated with infection by the respiratory (group 2) virus.

Definitive diagnosis of respiratory *Canine coronavirus* would likely be problematic in most clinical settings, contributing to the probable under-appreciation of this agent as a cause of respiratory disease in dogs. In the recently documented high prevalence of respiratory coronaviral infection in the United Kingdom, no coronaviruses were isolated (7); the diagnosis was based on retrospective serology and polymerase chain reaction (PCR) to detect nasal shedding. The "negative" culture results in that study (7) could be related to the timing of sampling or the choice of cells used for culture. Isolation of the closely related respiratory *Bovine coronavirus* is most successfully achieved in specific clones of a human rectal carcinoma cell line, which may not be routinely used in cases where isolation of canine respiratory viruses is attempted (10). Further studies employing paired serology are necessary to further implicate *Canine coronavirus* in cases of canine respiratory disease and should be considered as an approach in outbreaks of respiratory disease in dogs (9).

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