

## Immunohistochemical detection of canine distemper virus in haired skin, nasal mucosa, and footpad epithelium: a method for antemortem diagnosis of infection

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**Abstract.** A reliable antemortem diagnostic method is needed for determining infection with canine distemper virus (CDV). The utility of immunohistochemical detection of CDV antigen was examined for samples of nasal and footpad epithelium and haired skin in dogs with and without detectable CDV antigen in the lung and/or brain. Tissues from 57 dogs at risk of CDV infection were tested. Viral antigen was found in the lung and/or brain of 28 dogs. Among these dogs, viral antigen was demonstrated in the epithelial cells of the nasal mucosa in 24 of 27 dogs, in the footpad epithelium in 24 of 26 dogs, and in the haired skin of the dorsal neck in 26 of 27 dogs. Among the 29 dogs without CDV antigen in either the lung or brain, 1 dog had positive staining for viral antigen in the skin and nasal mucosa. Biopsies of haired skin of the dorsal neck, which is relatively simple to sample, can be used for antemortem immunohistochemical testing for acute and subacute infection with CDV.

Canine distemper virus (CDV) and related morbilliviruses are widespread, and infection with these agents continues to cause high-mortality epidemics in many species, including domestic dogs.<sup>9,13,18</sup> The post-mortem diagnosis of morbillivirus infection is relatively simple and reliable using immunohistochemical staining,<sup>4,7,14,16,17</sup> however, antemortem diagnosis remains problematic.<sup>6</sup> Although the virus is shed in many secretions and excretions, it difficult to isolate in cell culture.<sup>2,3</sup> Immunofluorescence detection of viral antigen in conjunctival scrapings,<sup>2,11,20</sup> epithelial cells in urine,<sup>15</sup> and mononuclear cells in the cerebrospinal fluid<sup>1,21</sup> is reported to be of variable but usually low sensitivity. Serology for CDV antibodies, especially in vaccinated animals or those with unknown vaccination histories, is difficult to interpret.<sup>8</sup> The recently described polymerase chain reaction method for detection of this virus<sup>19</sup> is not widely available and is of undetermined efficacy.

Recently, there have been a series of epidemics of CDV infection in dogs in western and northern Canada. Because CDV is known to replicate widely in a variety of epithelial and mesenchymal tissues,<sup>2,8</sup> the goal of this study was to determine if immunohistochemical staining of foot pad epithelium, nasal mucosa, or haired skin would accurately reflect the pres-

ence of systemic CDV infection and thus suggest that testing of biopsies of these tissues would be useful in antemortem diagnosis.

### Material and methods

*Dogs.* Tissue samples were obtained from 57 dogs; 35 dogs that were euthanized at the Saskatoon Society for the Prevention of Cruelty to Animals (SPCA) during an epidemic of CDV infection in the spring of 1996 and 22 individual pet dogs from which tissues were submitted during 1996–1997 to the Diagnostic Immunology Laboratory of the Western College of Veterinary Medicine (WCVM) for immunohistochemical testing for CDV infection. Many of the latter dogs were SPCA adoptees that developed respiratory or other disease symptoms following adoption.

*Histology and immunohistochemistry.* Tissue samples (approximately 1 cm<sup>3</sup>) of lung, nasal mucosa, and haired skin of the dorsal neck were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin wax. In 10 of the 22 pet dogs, samples of brain tissue were also examined.

Four 5- $\mu$ m sections of each tissue block were cut. For tissues collected from the 35 SPCA dogs, 1 section was stained with hematoxylin and eosin (HE) and examined by light microscopy. Tissues were examined for evidence of pneumonia (lung) and parakeratosis or hyperkeratosis (nasal mucosa, footpad epithelium, and skin) and the presence of structures consistent with inclusion bodies. HE-stained slides of the tissues collected from the 22 pet dogs were examined by diagnostic pathologists at the WCVM, and tissue sections were submitted for CDV immunohistochemical examination if the history of the animal and/or histologic lesions suggested CDV infection.

The remaining 3 sections from each tissue block were mounted on slides<sup>a</sup> coated with 0.1% poly-D-lysine<sup>b</sup> and im-

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**Table 1.** Detection of CDV antigen in 57 dogs at risk for infection with CDV using histologic and immunohistochemical (IHC) techniques.

CDV antigen in lung/ brain <sup>a</sup>	n	IHC for CDV antigen <sup>b</sup>		
		Nasal epithelium	Footpad epithelium	Skin
Positive	28	24/27	24/26	26/27
Negative	29	1/29	0/29	1/27

<sup>a</sup> Using immunohistochemical techniques.

<sup>b</sup> No. positive/no. tested.

munohistochemically stained for demonstration of CDV antigen. The immunohistochemical stain was an avidin–biotin complex technique<sup>12</sup> adapted for a robotic slide stainer<sup>c</sup> utilizing a rabbit polyclonal antisera to measles virus.<sup>d</sup> Two sections of each tissue block were stained with 1/4,000 and 1/8,000 dilutions of anti-CDV antiserum, and the third section was stained with a 1/4,000 dilution of an irrelevant rabbit antiserum. A section of brain tissue from a CDV-infected dog was stained simultaneously with each group of test slides. The evaluation of the footpad, nasal epithelium, and skin tissues was conducted without knowledge of the results of the testing of the lung and/or brain tissue. Immunohistochemical staining for CDV was evaluated as either positive or negative.

## Results

CDV antigen was found in the lung sections of 27 of 57 dogs. Among the 10 dogs in which brain was tested, viral antigen was found in 8, 7 of which also had antigen present in the lung. In 1 dog, the brain was antigen positive and the lung was negative.

Among the 28 dogs that were CDV antigen positive in the lung and/or brain, viral antigen was found in the nasal epithelium in 24 of the 27 dogs tested, in the footpad epithelium in 24 of the 26 dogs tested, and in the haired skin in 26 of the 27 dogs in tested (Table 1). In 1 dog, positive staining for CDV antigen was found in the nasal and skin epithelial cells but not in the lung or footpad.

The distribution of the positive staining for viral antigen varied from small focal areas comprised of a few epithelial cells to extensive staining of the majority of the epithelial cells in the section. In haired skin, the positive staining was most often found in hair follicle epithelium (Fig. 1a–c).

In 20 of the 35 dogs euthanized at the SPCA, there was histologic evidence of pneumonia that varied from mild interstitial to severe and suppurative. Ten of the dogs with pneumonia were positive for CDV antigen, and 10 were negative. Two of the 15 dogs without pneumonia had detectable CDV antigen in the lung section (data not shown). There was hyperkeratosis and/or parakeratosis of the nasal and/or footpad and skin epithelium in 26 dogs: 11 dogs with and 15 dogs

without detectable CDV antigen. Inclusion bodies were histologically apparent in 10 dogs, 7 of which were CDV antigen positive and 3 of which were negative (data not shown).

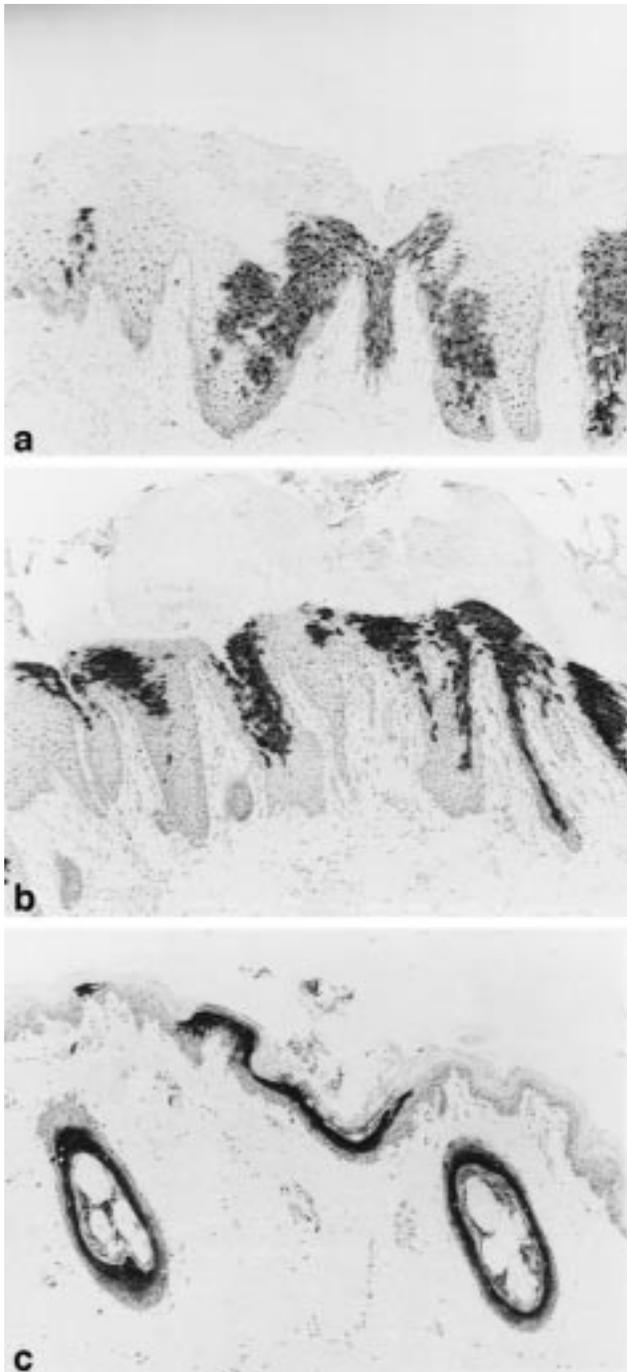
## Discussion

In the vast majority of cases in which CDV antigen was found in the lung and/or brain, viral antigen was also detected 1-cm samples of nasal epithelium, footpad epithelium, and the epithelium of haired skin of the dorsal neck, suggesting that biopsies of these tissues could be used to make an antemortem diagnosis of this infection. The advantage of haired skin compared with nasal mucosa and footpad epithelium is ease of antemortem sampling.

Histologic lesions, including the observation of inclusion bodies, were not a reliable indicator of CDV antigen detection. Similar observations have been previously reported.<sup>7,16</sup> Inclusion bodies appear relatively late in the course of the disease,<sup>11</sup> and other structures may be mistaken for virus-induced inclusions.<sup>5,22</sup> Many dogs, both with and without detectable CDV antigen, had histologic evidence of pneumonia. Many of the dogs in this study were abandoned or stray dogs housed in an animal shelter and might be at risk for infection with a variety of other agents causing respiratory disease, such as *Bordetella bronchiseptica* and parainfluenza type 5 virus. The observation that parand hyperkeratosis of the skin, footpad, and/or nasal mucosa was not a good predictor of the presence of CDV antigen is also not surprising because these lesions are not reliably present or specific for CDV infection.<sup>8</sup>

The presence of CDV antigen in the lung or brain was used to define positive cases of CDV infection. CDV infects multiple tissues; however, pulmonary infection, although variable in severity, is believed to be always present.<sup>2,8,11</sup> The virus is present, at least within the mononuclear cells in this tissue, prior to its spread to other cells and organs and persists after clearance from other visceral organs.<sup>2,8,10,11</sup> The early clinical signs most often associated with CDV infection (respiratory and gastrointestinal) develop about 8–10 days postinfection, and acute neurologic symptoms develop about 1 week later.<sup>2,8,10,11</sup> In 1 dog, viral antigen was found in a small number of cells only in the lung and not in the other tissues examined. This animal was an SPCA adoptee that presented with respiratory signs and was euthanized and may represent an early stage of CDV infection prior to the dissemination of the virus. Tissue from the brain of this dog was not available for testing.

Most dogs that acquire CDV infection die in the acute or subacute phases (up to 30 days postinfection) in which virus is present in many tissues; however, in



**Figure 1.** Immunohistochemical staining for CDV antigen in formalin-fixed, paraffin-embedded tissues. Avidin–biotin complex immunoperoxidase stain, diaminobenzidine as chromagen, hematoxylin counterstain. **a.** Nasal mucosa. **b.** Footpad epithelium. **c.** Haired skin of the dorsal neck.

a proportion of dogs there is either complete clearance of the virus and recovery from disease or clearance of the virus from tissue other than the brain.<sup>2,9</sup> The late neurologic syndromes associated with CDV persistence in the brain occur either 1–2 months after infection or manifest as a very rare syndrome of progressive

encephalitis from 24 months onward.<sup>10</sup> Among the animals tested in this study, the brain was examined in 10 dogs. In 7 of the 8 dogs in which the virus was present in brain, it was also found in the lung and other epithelial tissues tested. However, in 1 dog in which there was virus in the brain, no virus was found in the lung and only a few infected cells were found in the skin. This dog was a puppy presented with neurologic signs typical of CDV infection and may represent an instance in which virus has been cleared from non-neurologic tissues. It is unlikely that immunohistochemical staining for virus in epithelial cells of biopsy samples will be useful in advanced neurologic canine distemper cases because significant amounts of virus are not thought to be present in tissues other than nervous system. However, there is also evidence for persistence of CDV in the footpad epithelium; therefore, it might be useful to test this tissue in suspect animals.<sup>2</sup>

Among the 29 dogs that did not have CDV antigen in the lungs (or brain), other tissues were also negative in 28 dogs. In 1 dog, viral antigen was present in the nasal mucosa and in haired skin. Because the virus can be focally distributed in the lung and elsewhere, some cases of systemic infection may be missed with the relatively small amounts of tissue examined immunohistochemically.

The immunohistochemical test used on formalin-fixed tissues could also be applied to cryostat sections of frozen skin biopsies. Either frozen or fixed tissues can, depending upon the efficiency of the laboratory, be processed and stained for CDV antigen within 1 day of arriving at the laboratory, thus providing a rapid, sensitive, and specific method for confirmation of acute and subacute CDV infection.

### Sources and manufacturers

- a. Codon Slides, Fisher Scientific, Edmonton, AB.
- b. Sigma Chemical Co., St. Louis, MO.
- c. Codon Histomatic Stainer, Fisher Scientific, Edmonton, AB.
- d. Dr. B. Ziola, Department of Microbiology, University of Saskatchewan, Saskatoon, SK.

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