Circovirus Infections in Dogs

The newest emerging viral infection of dogs is canine circovirus. It was thought to be the cause of illness and mortality in Ohio dogs in late August/early September 2013. Canine circovirus can either be the primary infection or present in the form of co-infection with other enteric pathogens. Its role as a primary pathogen still needs to be defined. In a recent paper, Linlin et al (1) documented co-infection between canine circovirus in dogs with diarrhea and canine enteric coronavirus, Cryptosporidium spp, C. perfringens α toxin, Giardia spp, Salmonella spp, Campylobacter jejuni, or Campylobacter coli. Canine circovirus was detected in the feces of approximately 11% of dogs with diarrhea, but was also detected in 14 of 204 healthy dogs. Almost 70% of the dogs that tested positive for canine circovirus were found to be co-infected with at least one additional enteric pathogen.

Clinical signs associated with canine circovirus infection include progressive vomiting, diarrhea, which can be hemorrhagic, hematochezia, ascites, pleural effusion, hypovolemic shock, bicavitary hemorrhage and disseminated intravascular coagulation.

The main lesion in suspect dogs was found to be fibrinonecrotizing vasculitis, detected primarily in the intestine and kidneys, but also in urinary bladder, spleen, liver, lungs, heart, pancreas, adrenal glands and meninges. All dogs examined had lymphadenitis in Peyer’s patches. The laboratory identification of canine circovirus should be made by a combination of real-time PCR and another assay, such as immunohistochemistry or in situ hybridization, to demonstrate the presence of viral proteins or DNA within cells associated with the lesions.

Canine respiratory coronavirus

Infection with canine respiratory coronavirus is commonly detected in dogs with respiratory disease, but until recently its pathogenesis and the role it plays in the development of canine infectious respiratory disease (CIRD) were largely undefined. In the first recently published
pathogenesis study (2), experimental inoculation with each of 5 isolates from different geographical areas lead to respiratory disease signs which were consistent with those occurring during natural infection. Viral was consistently detected from oropharyngeal swabs until day 10 post-infection.

Histopathological changes were observed in the nares and trachea and consisted of inflammatory damage to tracheal cilia. The virus was re-isolated from a wide range of respiratory tissues, mucosal associated lymphoid tissues, and from lung lavage fluids. By day 14 post-infection, inoculated dogs had developed antibodies which were neutralizing both homologous and heterologous strains used in the study, thus pointing to antigenic homogeneity among strains from two continents. Laboratory diagnosis of canine coronavirus is made primarily by RT-PCR, since the virus is difficult to propagate in cell culture. Nasal swabs and tracheal washes are the most appropriate samples from live dogs Nasal tonsil, trachea and lung are suitable tissues to collect during postmortem examination.

**Canine distemper virus**

Although widespread use of effective vaccines has made canine distemper fairly uncommon in the US, outbreaks do occur from time to time. One of recent outbreaks (3) occurred in Wyoming during August–October 2010 and involved 24 young dogs of multiple breeds. Cases occurred shortly after sale of the dogs involved by 2 pet stores. The diagnoses were made based upon gross and histopathology, electron microscopy, direct FA and RT-PCR. Sequencing of PCR products demonstrated that the sequences from 2 dogs that were part of the outbreak were identical. The breeding property in Kansas from which the puppies originated was quarantined by the Kansas Animal Health Department and all puppies intended for sale were tested. Canine distemper was diagnosed at the Kansas facility in November 2010. The decision was made to euthanize 1,466 dogs to eliminate viral dissemination through sales.

Canine distemper virus strains with enhanced neuronal tropism, also leading to high mortality in wild carnivores were recently reported in Switzerland (4).

A canine distemper epidemic, first detected there in the spring of 2009, was characterized by unusually high morbidity and mortality, rapid spread all over the country, and susceptibility of several wild carnivore species. The main lesions consisted of interstitial to bronchointerstitial pneumonia and meningopoliencephalitis. Demyelination, which is typically associated with CDV infection, was observed in few cases only. In the brain, viral inclusions were mainly detected in neuronal nuclei. In vitro analysis of the hemagglutinin protein from one of the Swiss CDV strains revealed functional and structural differences from that of a reference strain, such as
increased cell surface expression of signaling lymphocyte activation (SLAM) receptors and enhanced viral binding efficiency to this receptor.

Periodically CDV infections also occur in zoos. Clarke et al. (5) described a canine distemper virus infection in a Siberian-cross tiger from an animal sanctuary in northeastern Mississippi. A 9-year-old spayed female tiger, which had been a resident at the sanctuary since she was 6 months old, acutely exhibited progressive paraparesis and myoclonus of the right hind limb. Raccoons exhibiting upper respiratory signs were observed entering the tiger enclosure. Clinical examination showed intact pupillary light and limb reflexes. Blood work was unremarkable. Spinal radiographs were normal but myelography revealed mild narrowing at C7-T1. A combined FeLV/FIV test was negative. CSF analysis revealed 96% moderate to large lymphocytes with occasional prominent nucleoli. Serology for toxoplasmosis, neosporosis, cryptococcosis and tick-borne disease was not definitive. The tiger was euthanized. Histopathology revealed nonsuppurative encephalomyelitis, microgliosis, spongiosis and mild leptomenigitis. Eosinophilic intranuclear and intracytoplasmic inclusion bodies were in neurons and astrocytes. Reverse transcription PCR (RT-PCR) detected viral RNA, both with nucleoprotein and phosphoprotein (P) gene-specific primers. Sequencing alignment of 540-bp P-gene fragment of the tiger strain with corresponding sequences from neurologic foxes and raccoons collected from northeastern Mississippi and diagnosed with Morbillivirus revealed that fox and raccoon strains differed by 1-bp. The tiger strain, however, had 7 or 8 base mismatches with the raccoon and fox, respectively. Thus, wildlife distemper strains circulating concurrently are identical, but unrelated to the tiger strain.

Canine pneumovirus

Canine pneumovirus has been isolated at Cornell University from the respiratory tracts of dogs in 2 related shelters (6)). Follow-up diagnostic testing yielded 3 new viral isolates and identified 6 additional PCR positive dogs from other USA locations, indicating that canine pneumovirus has a wider geographical distribution. Genetic analyses have shown that this virus shares sequence homology with pneumonia virus of mice. In a recent study, mice were experimental inoculated with canine pneumovirus. The virus was shown to replicate in airway and lung tissue of the experimental mice and elicited local pro-inflammatory cytokine production in these tissues. However, induction of fatal infection after inoculating the mice intranasal required very high-titered inocula. Experimentally inoculated mice that recovered had virus neutralizing antibodies in their sera that were cross-protective against pneumonia virus of mice (7).
**Canine influenza virus**

Canine influenza virus was first described in Florida greyhounds in 2004 and was associated with severe outbreaks of respiratory disease. Since then, canine influenza has been documented in 30 states and Washington, DC. At this time, CIV is endemic in areas of Colorado, Florida, New York, Pennsylvania, and Nevada. Genetic analyses of the initial isolate indicated that canine influenza virus is genetically very closely related to the H3N8 type of equine influenza virus. CIV has adapted itself through point mutations in the H gene to replication in the canine host. Recent experimental inoculations have shown that the virus is now genetically different enough from the original equine virus to no longer be transmissible to horses.

The clinical signs of canine influenza virus infection occur after an incubation period of only 2-3 days and the morbidity is very high. Most of the infected dogs present with the mild form, characterized by low grade fever, a cough that may last for up to 2 weeks and nasal discharge that is initially serous but can become mucopurulent following secondary bacterial infection. Approximately 5% of infected dogs experience the more severe form, which can be fatal, and is characterized by high fever. Necropsy findings associated with the severe form are extensive hemorrhages in the lungs, mediastinum, and pleural cavity.

Nasal swabs are the best sample for the laboratory diagnosis of CIV infections. It needs to be stressed that it is crucial to collect antemortem samples very early during the clinical course of the disease, and if possible also from asymptomatic dogs in contact with the animal showing clinical signs. Trachea and lung are appropriate postmortem samples. The virus has been isolated in cell culture, but the timing of the sampling is crucial. Infectious virus is no longer detectable by 7 days post-infection. Our laboratory is using a real time RT-PCR to detect viral RNA in nasal swab extracts. Serological testing is done by the hemagglutination inhibition (HI) test.

In the first published study investigating the prevalence of canine influenza virus infection in sled dogs, a total of 399 dogs racing in the 2010 Iditarod were tested for antibodies by the hemagglutination inhibition test (8). Surprisingly, none of the samples tested, including 39 samples from dogs reported as having been vaccinated against canine influenza, were seropositive by HI. All vaccinated dogs were also negative for virus neutralizing antibodies, justifying additional studies on both the prevalence of canine influenza infection and vaccine efficacy in this population. As indicated above, rapid and accurate detection of infection is critical to the diagnosis and control of canine influenza virus infection.

The first objective of a recently published study (9) was to compare the relative sensitivities of different laboratory methods on canine swab samples. The methods being compared were virus isolation, a commercial rapid antigen detection ELISA and real-time reverse transcription
polymerase chain reaction (RT-PCR). The second objective was to compare the performance of the RT-PCR and a lateral flow avian influenza A rapid test on samples collected from shelter dogs. The median sensitivities of virus isolation, rapid influenza diagnostic test, and real-time RT-PCR were calculated to be 72%, 65%, and 95%, respectively. The lateral flow avian influenza A antigen test was found to be significantly less sensitive than RT-PCR for detecting nasal shedding in shelter dogs.

**Feline leukemia**

A fraction of cats exposed to feline leukemia virus (FeLV) effectively contain the virus and effectively resist persistent antigenemia/viremia. By a real-time quantitative PCR approach to quantitate circulating viral DNA levels persistent FeLV DNA has been detected in blood cells of non-antigenemic cats which had been considered to have resisted FeLV challenge. In addition, the vast majority of the viral DNA detected was transcriptionally active, even in the absence of antigenemia.

To determine whether FeLV vaccination would prevent nucleic acid persistence, circulating viral DNA, RNA, antigen, infectious virus, and virus neutralizing (VN) antibody in vaccinated and unvaccinated cats challenged with infectious FeLV were recently determined. Challenged vaccinates with undetectable antigenemia and viremia concomitant with persistent FeLV DNA and/or RNA were identified in this study. Two whole inactivated virus (WIV) adjuvanted FeLV vaccines provided effective protection against FeLV challenge (10, 11).

**References**


