It's Distemper, Dog Gone It!

A Case of Canine Distemper Virus in a Raccoon

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Introduction

Canine distemper virus (CDV) is a highly contagious disease that affects domestic dogs and multiple wildlife species. Wildlife species, particularly procyonids (raccoons, coatis, olingos), can serve as reservoir hosts to domestic dogs.[2] Clinical signs can be nonspecific initially and can progress to life threatening symptoms. CDV is a virus of significance due to its high virulence and threat to susceptible endangered species. Identification of the disease and prevention of future infections are key to reducing the prevalence of CDV in the United States.

History and clinical signs

On April 25, 2018, an approximately 6-month-old intact female raccoon presented to Mississippi State University College of Veterinary Medicine (MSU-CVM) Laboratory Services for necropsy after euthanasia that morning. The raccoon was found in a Starkville subdivision. She was walking in circles, falling over, and disoriented. No ocular discharge was noted in either eye on presentation.

Necropsy findings

On gross examination, the raccoon had a body condition score of 2/9. There were multiple ticks in the fur (acariasis). The trachea had reddened gelatinous material as well as red to brown frothy foam in the distal portion, cranial to the mainstem bronchi. There was dark red to brown frothy foam in the mainstem bronchi. The frothy foam was more concentrated in the right side compared to the left. The right lung was darkened red to purple, heavy, and firm primarily within the caudal and dorsal aspects. The cranial and ventral portion of the right lung was firm and pink. The left lung was relative normal. It was reddened primarily within the caudal and dorsal aspects. Approximately 5% of the cranial area of the left lung was firm and pink. The heart valves were smooth and shiny. There was no evidence of fluid within the pericardial sac.

The brain, pituitary, eyes, thyroids, and lymph nodes had no gross lesions. The spleen was contracted and split into two pieces. The liver had 2 focal areas with white string like material that attached to the omentum. The focal areas on the liver were tan, smooth, and approximately 5mm in diameter (tension lipidosis). The stomach had dark brown, muddy contents within it. The intestines, pancreas, kidneys, bladder, and adrenal glands had no gross lesions visible.

All tissues were submitted for histopathology. The brain had marked spongy changes in the white matter of the internal capsule, optic chiasm, and cerebellum. Axonal spheroids and digestion chambers were frequently present, with gitter cells and reactive glia. The optic chiasm was severely affected. There was a focal group of deep cortical neurons and astrocytes that contained many intranuclear and intracytoplasmic magenta inclusions. Foci of gliosis and perivascular cuffing were associated with inclusion bodies. Both eyes had retinal changes. One eye had mild lymphocyte and plasmacyte infiltration of the ganglion layer. The other eye had severe retinal atrophy with degeneration and scarring, loss of nuclear layers, and foci of melanophages. There was abundant inflammation within the lungs associated with the airways and the surrounding alveoli. The bronchi were filled with pink fluid, and the epithelium was irregular, varying from tall, well differentiated to shorter, simple columnar, variably ciliated epithelium that lacked goblet cells. Bronchial glands were ectatic with attenuated epithelium. Bronchioles were lined by hyperplastic cuboidal epithelium which exhibited irregular attenuation with few sloughed cells and karyorrhectic debris. Type 2 pneumocyte hyperplasia lined the surrounding alveoli, which contained foamy macrophages, few heterophils, and pink fluid. The stroma was expanded by collagenous tissue and lymphocytes with macrophages. The inflammation tended to encircle veins. The tracheal epithelium had fewer ciliated cells. There were mildly increased numbers of lymphocytes and granulocytes between epithelial cells. A

small number of cortical tubules in the kidneys were dilated by hyaline pink fluid with few sloughed cells. Small clusters of lymphocytes and macrophages with few heterophils were present at the corticomedullary junction, with mild interstitial fibrosis. Few epithelial cells in the kidneys contained large hyaline pink inclusion bodies. The liver had diffuse delicate tracts of dense collagen bridging triads and central veins. There was mild biliary and arteriolar hyperplasia, with scant chronic portal inflammation present. Siderophages were common throughout the liver. Kupffer cells contained abundant fine brown granular pigment. Sinusoids contained many leukocytes of various lineages, plump Ito cells with lipid vacuoles, and small numbers of apoptotic cells. Hepatocytes contained abundant fine brown granular pigment and exhibited cloudy swelling with lipid vacuoles. All other histological findings were not pertinent for the diagnosis of this raccoon.

In this case, CDV was diagnosed through gross lesions and histopathology findings. The brain revealed severe, chronic, multifocal meningoencephalitis with demyelination and necrosis. Intracytoplasmic and intranuclear neuronal and astrocyte viral inclusions were also identified. Lymphoplasmacytic retinitis was diagnosed in both eyes with retinal degeneration and scarring. The lungs had a severe chronic bronchointerstitial pneumonia. The kidneys had mild, chronic, multifocal mixed nephritis with intraepithelial viral inclusions and mild proteinuria. Moderate, chronic, non-specific hepatitis with fibrosis was diagnosed in the liver.

Pathophysiology

Canine distemper virus (CDV) is a morbillivirus belonging to the Paramyxoviridae family. CDV is an enveloped single stranded RNA virus that has six structural proteins, similar to other paramyxoviruses. The proteins are termed: nucleocapsid (N), phospho (P), large (L), matrix (M), hemagglutinin (H), and fusion (F).[1] Mutations of the H protein are common and are

associated with CDV's virulence and ability to adapt to a wide variety of hosts.[1,2] Protein H is the viral attachment factor that induces the majority of CDV neutralizing antibodies. Infection is achieved when the H protein of CDV binds to the signaling lymphocyte activation molecule (SLAM) receptors within the host's cells. This activation leads to a fusion of the virus's envelope and the host cell membrane which in turn activates neighboring cells, leading to cell lysis.[2] Activation of SLAM receptor and nectin-4 receptor allow CDV to bind to the surface of host cells. SLAM is an immune cell receptor found on activated T and B lymphocytes, dendritic cells, and macrophages. Nectin-4 is located on epithelial cells. The receptor is involved in cell adhesion, organization, and acts as an exit receptor after the virus is released from the host's cells.[4]

The most common route of infection is oronasally, however transmission can occur by other routes such as through urine or ingestion of infected meat. Within 24 hours post infection, the virus replicates in lymphoid tissue of the respiratory tract, specifically tissue macrophages.[3,6] The incubation period is variable and is between one to four weeks.[3] CDV uses the host's mucosal surfaces to disseminate throughout the lymphatic system and activate the SLAM receptors. Within 2 to 4 days, the virus replicates within the tonsils and retropharyngeal and bronchial lymph nodes. The virus disseminates to the spleen, stomach, small intestines, mesenteric lymph nodes, and Kupffer cells within the liver after 4 to 6 days post infection.[6] CDV infection has a biphasic fever viremia. The first phase, between days 3 to 6 post infection, primarily leads to immunosuppression through leukocyte necrosis and apoptosis. Lymphopenia, lymphoid depletion, and transient fever also occur within the first phase.[1,6] Immunosuppression can lead to secondary infections that will complicate the disease.[3] The second phase of CDV involves a high fever and a hematogenous spread of the virus to almost all organ systems.[1,6]

CDV can be found in the epithelium and central nervous system (CNS) tissues by day 8 to 9 post infection, depending on the host's humoral and cell mediated immune status. The virus can now be shed once it reaches the epithelium. If the host's immune system is adequate, the virus is cleared by day 14 post infection. Domestic dogs and other wildlife hosts can have an adequate immune system to clear the disease, but the virus can persist in the uvea, neurons, and skin, primarily in the footpads. After the host recovers from the CDV infection, long term immunity is achieved. This immunity can be compromised if exposed to another virulent strain or if the host becomes immunocompromised. [6]

CDV continues to spread to multiple tissues within dogs and other wildlife species that do not have the immune system capable of clearing the virus by days 9 to 14 post infection. These tissues include skin, endocrine and exocrine glands, and epithelium lining the gastrointestinal, respiratory, and urinary tracts. The clinical signs are generally severe at this stage of the disease and CDV will persist in the tissues until death.[6]

CDV spreads to the CNS either hematogenously or through neural routes. The virus enters the brain parenchyma through blood vessels and remains within the perivascular spaces. The virus can be identified in astrocytes and neurons. The virus also enters the choroid plexus of the fourth ventricle and replicates. The virus can reach cerebrospinal fluid (CSF) and spread to the optic tracts, cerebral peduncles, and the spinal cord.[3,6] CDV that is spread through the neural route disseminates to the olfactory neurons and replicates in the respiratory mucosal epithelium. The virus then passes through the cribriform plate and spreads to the olfactory nerve. Polioencephalomalacia rarely occurs through the spread of the virus to the pyriform and temporal lobes of the brain. Lesions within the CNS depend on the course of infection, immune status of the host, and virulence of the strain of CDV.[6]

Acute CDV encephalitis occurs in young or immunocompromised animals. It occur early in the course of infection and is characterized by direct injury to the CNS. Multifocal lesions within the grey and white matter are the result of neuronal infection and necrosis. These lesions can lead to polioencephalomalacia. Since these lesions occur early within infection, inflammation is minimal. Acute noninflammatory demyelination occurs within the white matter and is associated with replication in microglial and astroglial cells.[3,6]

Subacute to chronic CDV encephalitis occurs when there is a strong inflammatory response and a reduced CDV antigen expression. This inflammatory response is virus independent. Since this encephalitis occurs late in infection, the animals are recovering from lymphoid depletion. The virus is present mainly within the follicular dendritic cells of the lymphoid tissue. Proinflammatory cytokines are upregulated, which induces a strong humoral immune response. CDV infected macrophages within the CNS release reactive oxygen radicals, which destroy oligodendroglial cells and myelin. The virus itself is not the cause of demyelination in this case. The reaction of the immune system to the virus is what causes the damage. This damage can be fatal to the host.[1,6]

Old dog encephalitis is a rare, chronic, inflammatory disease of the grey matter of the CNS. This occurs when an immunocompetent animal becomes infected, but the virus continues to persist within the neurons. Inclusion body encephalitis can occur after vaccination or in animals that only have the neurologic form of CDV. Grey matter necrosis, inflammation, and intranuclear and intracytoplasmic viral inclusions are present.[6]

Diagnosis/treatment

CDV can be diagnosed in a variety of ways. Clinical signs must be differentiated from other infections, including primary CNS, gastrointestinal, and respiratory diseases. There are

multiple ways to identify CDV infection after obtaining a thorough history, including vaccination status. Immunofluorescence assays (IFA) can be used on antemortem specimens such as conjunctival scrapings, buffy coat, urine, cerebrospinal fluid, and foot pad biopsies to detect CDV inclusion bodies. IFA is most reliable within the first three weeks of infection and inclusion bodies within the CNS can be detected for more than 60 days post infection.[2] IFA or immunohistochemistry (IHC) can also be used to identify CDV in post mortem specimens.[2,4] Serology can be used to detect IgM and IgG antibodies. An elevated IgM titer identified by enzyme linked immunosorbent assay (ELISA) cannot differentiate between a recent infection or recent vaccination. The elevated titers can last up to 3 months, so this method's results can be misleading if the animal has been recently vaccinated. A four-fold increase in IgG antibody titers taken 14 days apart can indicate a CDV infection. The greater the increase in IgG titers, the most likely the animal is infected with CDV, even in recently vaccinated animals. Antibodies identified in CSF are highly indicative of a CDV infection since antibodies induced by the CDV vaccine do not cross the blood brain barrier. Virus isolation can take up to three weeks, however, newer cell lines expressing the SLAM receptor have shown results within a few days. Vaccination with a modified live vaccine can interfere with reverse transcription-polymerase chain reaction (RT-PCR) results. The results from RT-PCR can take up to four weeks. RT-PCR can yield a false negative, which does not rule out CDV infection, or a false positive due to recent vaccination status.[2] The serum neutralization test is commonly used in wildlife to detect CDV from serum samples because it is highly sensitive and specific. Serum neutralization cannot distinguish natural infection from the virus strain used in vaccines, but ELISA testing kits are not always available for wildlife species.[4]

Treatment of CDV is difficult, especially in wildlife populations. Supportive and symptomatic therapy is primarily used in domestic dogs.[4] Intravenous fluids may be warranted in patient with anorexia and diarrhea. Antibiotics may be warranted if there is a secondary bacterial infection present, since CDV is highly immunosuppressive. Anticonvulsants can be used as symptomatic therapy to control seizures when CDV reaches the CNS.[5] There is no specific antiviral therapy to use against CDV infection.[4]

Current *in vitro* antiviral studies are being performed in ferrets to evaluate an orally available pan-morbillivirus inhibitor that targets viral polymerase. Treatment with the viral inhibitor at the onset of viremia has shown to reduce viral loads and clinical signs, ultimately allowing ferrets to recover from infection. Other *in vitro* studies on a polysaccharide known as fucoidan found in brown algae has shown antiviral properties against CDV infection. Fucoidan inhibits the initial viral replication as well as preventing binding to the host cells. Flavonoids, phenolic acids, mesenchymal stem cell therapy, and silver nanoparticles have also been researched to aid in the treatment of CDV infection.[4]

Prevention/control

CDV is a worldwide disease that can be fatal in both wildlife and domestic dogs.[1] Domestic dogs are the main reservoir of CDV, however it has been identified in many wildlife species, with the raccoon being one of the most common reservoir. Spillover can occur from either wildlife to domestic dogs, or vice versa.[1,2] The spillover prevents CDV from being eradicated, so prevention of the spread of disease is vital to avoid outbreaks.[1] Vaccination of domestic dog populations can help control CDV infections, but vaccination of 95% of the population is needed to control the virus.[2] Both modified live virus vaccine and recombinant canarypox vectored CDV vaccines are recommended by the American Animal Hospital

Associated Canine Vaccine Guidelines. The recombinant vaccine is more likely to produce antibodies in puppies with maternal antibodies still present. The recommendation is to give 3 series of vaccinations beginning at 6 to 16 weeks of age, followed by a booster at 1 year of age, then every 3 years after. Unvaccinated puppies and infected dogs should be isolated to avoid any exposure to any other dogs. Disinfection of areas that have been exposed to CDV is vital to prevent the spread of infection. CDV can be spread via aerosolization, fomites, urine, and feces. CDV is inactivated by quaternary ammonium compounds or 70% ethanol solution within 10 minutes at room temperature.[2] The virus is also sensitive to UV radiation, heat, and dessication.[4]

The best methods to prevent the spread of CDV from domestic dogs to wildlife is to avoid interaction between animals that may carry the virus and to vaccinate domestic dogs. Wildlife populations are not vaccinated against CDV unless state or federal authorities determine that endangered species may benefit from vaccination. Oral bait vaccines used for diseases such as rabies and plague are not currently available for CDV. This method of mass vaccination is problematic for CDV because the mucosal immune response is unknown and maternal antibodies are difficult to overcome.[2] There are many challenges with vaccinating wildlife against CDV, such as safety and efficacy, route of vaccine delivery, logistics of administration of boosters, and the cost of the vaccination program.[4] CDV outbreaks are monitored through the U.S. Department of Agriculture- Animal and Plant Health Inspection Services (USDA-APHIS) Wildlife Services, which administers the National Wildlife Disease Program. This program is used as surveillance to record outbreaks of many diseases, including CDV.[2]

Prognosis

Prognosis of CDV in domestic dogs and wildlife vary based on the strain of the virus and the individual's immune response.[5] In naïve species, unvaccinated or susceptible populations,

CDV can be fatal.[2] Animals exhibiting CNS signs may recover or clinical signs could continue indefinitely. CDV has a mortality rate of approximately 50% in domestic dogs, with death occurring between 2 weeks to 3 months post infection.[2,5]

Conclusion

Canine Distemper Virus is a disease of worldwide concern, especially to endangered species that are susceptible to it. This case demonstrates how common the disease is in wildlife and how the clinical signs can be mistaken for other diseases. Since there is no specific antiviral treatment, CDV can be a fatal disease. The virus's ability to adapt to other hosts makes eradication efforts unsuccessful. The main ways to prevent the spread of disease is to vaccinate domestic dogs and to prevent interaction with potentially infected wildlife.

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