Canine Distemper Virus in a 3.5 Month Old Puppy

Kyle Walker

Clinical Advisor: Dr. Meghan Slanina

Basic Science Advisor: Dr. Emma Davies

Senior Seminar Paper

Cornell University College of Veterinary Medicine

October 7th, 2015

Ok for e Comment
Mr Smith

Abstract:

A 3.5 month old male Rottweiler presented for a one day history of seizures. The puppy had been previously vaccinated for canine distemper virus and tested negative for canine distemper virus one month prior to presentation. The puppy presented with an obtunded to stuporous mentation, left head tilt, tetraparesis and ataxia, absent menace and miotic pupils OU, mild positional rotary nystagmus, positional left strabismus, delayed proprioception in all limbs, and significant head and cervical pain. Neurolocalization indicated a multifocal lesion involving the forebrain and brainstem. An emergent MRI revealed diffuse severe pachymeningeal contrast enhancement and a cerebrospinal fluid analysis revealed a mixed cell pleocytosis including some eosinophils that cultured negative. Bloodwork, urinalysis and culture were unremarkable and infectious disease titers were submitted. The prioritized differentials for the patient included an infectious or auto-immune meningoencephalitis and treatment was initiated. Despite treatment, the patient's neurologic status declined to the point where his mentation was stuporous to comatose and he developed hyponatremia due to syndrome of inappropriate ADH. The patient became hypothermic and was euthanized. Postmortem diagnostics were positive for a canine distemper virus infection. This report will describe the clinical findings, diagnostics, and treatment in a dog with canine distemper virus and exploring the possibility of a vaccine induced infection.

Introduction:

Canine distemper virus is a viral infection that affects most carnivores (5). While it is fairly common in wildlife, only sporadic outbreaks are noted in domestic dogs (5). The most common group of individuals affected are young unvaccinated animals from 12-16 weeks old (2). Canine distemper virus is often an acute to subacute febrile disease that is fatal (2). There are respiratory, gastrointestinal, and central nervous system (CNS) manifestations that have been well documented. The patient in this report presented first with respiratory disease, which tested negative for canine distemper virus, and then again a month later with CNS disease. Postmortem diagnostics indicated infection with canine distemper virus as the cause of clinical signs. Based on the patient history of being isolated as a puppy and vaccinated, a natural infection was less likely and a vaccine induced infection was prioritized with the final genetic analysis results still pending. The objective of this paper is to examine the process involved in a thorough case workup from case presentation to preparing differential diagnoses. This case highlights the fact that obtaining an antemortem diagnosis in central nervous system diseases can be challenging and repeated testing is warranted for some disorders.

Case History:

The patient in this report was a 3.5 month-old intact male Rottweiler. The patient was obtained from a breeder in Pennsylvania on February 28th, 2015. He was an indoor only dog, lived with two other dogs and two cats that were vaccinated and healthy, and isolated from additional animals. The patient's pertinent vaccine history included receiving his first two canine distemper vaccines on February 5th, 2015 and February 26th, 2015 exactly at six and nine weeks of age. The vaccine the patient received was the Zoetis Vanguard Plus 5 vaccine. This vaccine is intended for healthy dogs six weeks of age or older and aids in providing protection against

canine distemper virus, canine adenovirus type 1 and 2, canine parainfluenza virus, and parvovirus (12).

The patient first presented to his primary veterinarian on March 6th, 2015 for diarrhea. A stool sample revealed coccidia and campylobacter organisms and a macrolide, tylosin, and albon (sulfadimethoxine) were prescribed as treatment. Bilateral otitis was also diagnosed and mometamax (gentamicin/mometasone/clotrimazole) and malaseb flush (2% miconazole and 2% chlorhexidine) were prescribed as treatment.

On March 20th, 2015 the patient developed a nonproductive cough at home. He was monitored at home until he was scheduled to receive his third canine distemper vaccine the following day. On presentation to the primary veterinarian on March 21st, 2015 for vaccination the patient was lethargic, had loose stool, and had a hacking cough that had increased in frequency over the past 24 hours and was now productive. The patient did not receive his third canine distemper vaccine and diagnostics were performed. A parvovirus SNAP test was performed due to the history of gastrointestinal signs and was negative. A compete blood count (CBC) and chemistry panel were submitted that revealed a microcytic, normochromic nonregenerative anemia likely physiologic attributed to his young age. At this time radiographs revealed an alveolar pattern in the right middle, right caudal, and left caudal lung lobes. No treatment was administered and the patient was referred to an emergency clinic with a presumptive diagnosis of pneumonia. The patient was then referred to the Cornell University Hospital for Animals (CUHA) Emergency Service due to the lack of appropriate facilities to deal with a potential infectious respiratory condition at the first emergency clinic.

The patient presented to the CUHA Emergency Service on March 21st, 2015 for pneumonia and lethargy and was hospitalized with the Internal Medicine Service for five days. A

CBC and chemistry panel revealed a normocytic, microchromic nonregenerative anemia likely physiological attributed to young age and a neutropenia likely due to sequestration in the lungs associated with inflammation from the pneumonia. A tracheal wash revealed a noninfectious suppurative inflammation and cultured negative. A canine influenza and canine distemper virus PCR were submitted and the results were both negative. In general a positive PCR result is indicative of infection and a negative result can result from many factors including improper sample handling or transportation (5). The patient was diagnosed with bacterial bronchopneumonia based on his clinical signs and the appearance on radiographs. Treatment was initiated that included unasyn (ampicillin/sulbactam), ceftazidime, doxycycline, fluids, nebulization, and coupage. The patient improved in hospital and was discharged on oral clavamox (amoxicillin/clavulanic acid) and doxycycline for the bronchopneumonia and clotrimazole solution for concurrent bilateral yeast otitis that was diagnosed in hospital.

On April 14th, 2015 the patient had a recheck appointment with his primary veterinarian. His clinical signs and radiographic signs were improved and the primary veterinarian discontinued clavamox (amoxicillin/clavulanic acid) and doxycycline and started the patient on azithromycin. Routinely the prescribed antibiotic treatment for pneumonia is discontinued two weeks after resolution of radiographic signs. The owners noticed in the few days following the treatment change that the patient appeared lethargic and painful so they administered a dose of carprofen.

On April 20th, 2015 the patient seemed unaware of his surroundings, was lethargic, and was panting so the owners brought him to the CUHA Emergency Service. On presentation neck pain was elicited and bloodwork and cervical neck radiographs were unremarkable. The cervical neck radiographs were performed to identify possible causes of the patient's clinical signs

including vertebral fractures or luxations, congenital skeletal malformations, etc. Also on repeat chest radiographs, the pneumonia appeared markedly improved compared to the set of chest radiographs performed on March 21st, 2015 with only a mild diffuse bronchial pattern remaining. A suspected cervical soft tissue injury was diagnosed and the patient was discharged with tramadol for the pain. Overnight at home the patient experienced two seizure-like episodes (chomping and foaming at the mouth) and was brought back to the CUHA Emergency Service on April 21st, 2015. On presentation the patient had a stuporous mentation so an emergent MRI and cerebrospinal fluid (CSF) analysis were performed by the Neurology Service.

Clinical Findings:

On presentation the patient's mentation was changing between the initial stuporous mentation and an obtunded mentation. The majority of his vital parameters were within normal limits with a heart rate of 90 beats per minute and a temperature of 102.5 degrees Fahrenheit. He was tachypneic with a respiratory rate of 40 breaths per minute, likely due to stress or pain. On auscultation of his lungs, bronchovesicular sounds were heard in all four lung fields and paired with the improvement of radiographic signs suggested a resolving bronchopneumonia.

On neurologic examination the patient was obtunded to stuporous, exhibited a left head tilt, was non-ambulatory tetraparetic, had an absent menace and miotic pupils OU, positional rotary nystagmus, positional left strabismus, delayed proprioception in all limbs, and severe head and cervical pain. In addition, the patient was intermittently having simple partial, complex partial, and generalized seizures.

Problem List:

Following the neurologic examination a problem list was generated for the patient. The neurologic examination results made neurolocalizing the patients lesion to one area complicated.

Seizures localize to the forebrain and occur as a result of a neurotransmitter imbalance leading to spontaneous depolarization of a mass number of neurons (8). Menace deficits can result from cranial nerve two (optic nerve) lesions which can involve the optic nerve, optic chiasm, lateral geniculate nucleus, and/or visual cortex localizing to the forebrain (8). Menace lesions can also be attributed to cranial nerve seven (facial nerve) lesions in the medulla in the brainstem, cerebellar lesions, and are a common postictal sign (3). Mentation changes commonly associated with forebrain lesions are obtunded to stuporous, as seen in this patient. Mentation changes can also result from brainstem lesions and frequently do when patients are severely affected (3).

A left head tilt can result from peripheral cranial nerve eight (vestibulocochlear nerve) lesions or vestibular nuclei lesions in the medulla in the brainstem (3). In addition, the positional strabismus and rotary nystagmus noted are associated with a vestibular localization. Given the mentation changes and delayed proprioception the lesion was considered central. Tetraparesis, ataxia, and delayed proprioception deficits in all limbs are due to loss of upper motor neurons to all the limbs and localize to a brainstem or cervical spinal cord lesion (3). Miotic pupils can be attributed to loss of sympathetic fibers to the eyes resulting from a lesion anywhere but the forebrain, cerebellum, and caudal to the cervical vertebrae or cranial nerve three (oculomotor nerve) nucleus lesions in the midbrain in the brainstem (8). Head and cervical pain are nonspecific findings localizing to either the forebrain, brainstem, or cervical spinal cord (3).

After taking all the neurologic findings into account, the patient's lesion was neurolocalized as a multifocal lesion including the forebrain and brainstem. Cerebellar and cervical spinal cord lesions were ruled out due to the lack of associated signs. The problem list now included a multifocal lesion, seizures, pain, and a resolving bronchopneumonia. The multifocal lesion was the main problem focused on in this patient. The seizures and pain were

most likely secondary problems and the bronchopneumonia was resolving based on clinical and radiographic signs.

Differential Diagnosis:

From the problem list, differential diagnoses were formulated that prioritized a multifocal, progressive disease in a painful puppy. Degenerative, metabolic, and toxic causes of the patient's neurologic signs were considered less likely because they are not commonly associated with pain. Trauma was unlikely given the progression of clinical signs and absence of trauma in the patient's history. Anatomical causes included hydrocephalus, although this would not explain the patient's previous pneumonia. Neoplasia is less likely in younger patients; however, some neoplasias such as lymphoma and medulloblatoma can occur in young dogs (8).

The last two categories considered were infectious and inflammatory and were prioritized at this time due to the previous pneumonia, progression, and young age of the patient. The possible infectious causes included bacterial (Staphylococcus, Streptococcus, Proteus, E. coli), viral (canine distemper virus), fungal (Cryptococcus), parasitic (Cuterebra), protozoal (Toxoplasma, Neospora), and rickettsial (Rocky Mountain Spotted Fever) agents (8). It is important to note that canine distemper virus was lower on the differential diagnoses list due to the negative PCR result obtained from the Internal Medicine Service on March 21st, 2015. The inflammatory diseases considered at this time were auto-immune diseases such as granulomatous meningoencephalitis, necrotizing meningoencephalitis, necrotizing leukoencephalitis, and steroid responsive meningitis arteritis (8).

Diagnosis:

In order to rule the above differentials up or down on the differential diagnoses list, further diagnostics were required. The emergent MRI performed on presentation revealed a

diffuse, severe pachymeningeal contrast enhancement in the neurocranium. The neurocranium refers to the cerebrum and cerebellum and pachymeningitis is a thickening of the outer most layer of the meninges called the dura mater (8). This layer displayed contrast enhancement due to leakage into just the dura mater opposed to the other two layers of the meninges which are the arachnoid and pia mater (8). Nonsignificant findings included abnormal CSF intensity in the neck thought to be artifact and questionable cerebral and cerebellar atrophy. There was no evidence of lesions within the cerebral or cerebellar parenchyma.

The CSF analysis revealed a mixed cell pleocytosis including some eosinophils. The pleocytosis, or increased cell count in the CSF, consisted mainly of reactive lymphocytes. The eosinophils in the CSF could have been consistent with a breed-related steroid responsive condition or an infectious etiology, including a protozoal or fungal infection. The total protein was elevated at 196 mg/dL. The CSF was submitted for culture and cultured negative preliminarily. A coagulase negative Staphylococcus was isolated from enrichment; however, this was most likely due to contamination. The CSF analysis findings were consistent with an inflammatory process, with both an infectious and auto-immune process possible.

A CBC and chemistry panel were resubmitted upon presentation to the neurology service. The results were relatively unremarkable besides a mild normocytic, microchromic regenerative anemia of an unknown mechanism and a leukopenia most likely due to an acute infection or lymphocytolysis associated with a viral infection. A urinalysis and urine culture were submitted and were within normal limits and revealed no growth. At this time infectious disease titers were submitted for Neospora (IFA), Cryptococcus (serology antigen), Toxoplasma (IgG/IgM), and Rocky Mountain Spotted Fever (IFA). These results eventually all came back negative; however, some of the results were not available until after the patient was euthanized.

Final Outcome:

The patient's updated problem list now included meningoencephalitis, seizures, pain, and a resolving pneumonia. Meningoencephalitis was the main problem focused on and is defined as inflammation of the brain (encephalitis) and meninges (8). The evidence of pachymeningitis on the MRI, pain in the patient, and mentation changes were attributed to meningitis. The seizures the patient was experiencing indicated forebrain involvement, or encephalitis, even though abnormalities in the parenchyma were not appreciated on the MRI. The updated differential diagnoses for meningoencephalitis now excluded congenital and neoplastic causes based on the diagnostic results thus far. The prioritized differentials for meningoencephalitis included infectious or inflammatory causes as previously mentioned above. Several infectious diseases were ruled down as negative infectious disease titers returned; however, an infectious etiology could not be excluded as only the more common infectious agents were tested for and diagnostics are no 100% sensitive or specific. Auto-immune encephalitis remained a possibility as there is no definitive way of ruling this disease process in or out without a biopsy or histopathology.

Treatment:

The patient was treated for both an infectious and auto-immune meningoencephalitis. The initial treatments included supportive care consisting of fluids (Plasmalyte + 15mEq/L KCl) at 70ml/kg/day which is a little less than puppy maintenance (90ml/kg/day). Anticonvulsant therapy to control the seizures included a multi drug approach consisting of levetiracetam (22-37mg/kg IV q 8hrs; 22mg/kg IV for active seizure), phenobarbital (3mg/kg IV q 12hrs), and zonisamide (15mg/kg PO q 12hrs). Prednisone was used at an anti-inflammatory dose (0.5mg/kg PO q 12hrs) to decrease inflammation. Analgesic therapy to control the pain included fentanyl

(3mg/kg/hr CRI) and pregabalin (2mg/kg PO q 12hrs). The antibiotic choices in this patient targeted treatment for a protozoal, rickettsial, or bacterial meningitis. Enrofloxacin (10mg/kg IV q 24hrs) and ampicillin/sulbactam or unasyn (21mg/kg IV q 8hrs) targeted a bacterial etiology, doxycycline (7mg/kg PO q 12hrs) targeted a rickettsial etiology, and clindamycin (22mg/kg PO q 12hrs) targeted a protozoal etiology.

The patient did not show improvement through the first 36 hours of treatment. Cytarabine, a chemotherapy medication also known as cytosar (400mg/m^2 over 18-22hrs), was added to target the possible auto-immune causes more aggressively. On the third day of treatment, the patient showed a mild response to cytarabine and became less obtunded and mildly ambulatory, though he showed cerebellar signs (titubation and worsening head tilt). By the fourth day of treatment, the patient's mental status regressed back to severe obtundation bordering on stupor and the patient developed a downward trending hyponatremia (135-122.8mmol/L) most consistent with syndrome of inappropriate ADH. Attempts to correct the patient's sodium were unsuccessful. Despite brief improvement, the patient's neurologic status declined to the point where he was stuporous to comatose by the fifth day of treatment. Finally, the patient became hypothermic (96.8 degrees Fahrenheit) and was euthanized at the end of the fifth day of treatment.

Necropsy:

The owners elected necropsy after euthanasia. The final histopathologic diagnosis was lymphocytic meningoencephalitis which was consistent with the patient's clinical picture. It is interesting to note, right atrioventricular valve dysplasia and fibrinoid necrosis and neutrophilic arteritis of the intramyocardial coronary arteries were also diagnosed postmortem. The fibrinoid necrosis of the arteries is often a consequence of a type three hypersensitivity reaction induced

by vaccination, neoplasia, chronic infection, or a drug reaction. The neutrophils implies a bacterial infection, although no bacteria were observed. It is possible that rickettsia, mycoplasma, and/or bartonella organisms are responsible for this particular finding and were not seen because they do not stain on hematoxylin and eosin stain (H&E stain).

During the necropsy, samples were collected for postmortem diagnostics. Immunohistochemistry was performed on sections of cerebellum and basal ganglia, and on cerebellum and brain stem. The results were positive for canine distemper virus. Virus isolation was also performed and canine distemper virus was isolated successfully. Canine distemper virus was thought to be ruled down with the negative PCR result obtained on March 21st, 2015. Since the patient was isolated as a puppy and received his first two vaccines on schedule, a natural infection by canine distemper virus was less likely, although still possible. This prompted the consideration of a vaccine induced canine distemper virus. The isolated RNA sample was sent to Zoetis for sequencing against their vaccine strain to rule in or out vaccine induced canine distemper virus. The results of this test are still pending.

Discussion:

A brief review of canine distemper virus is required to understand the final diagnosis in this case. Canine distemper virus is a morbillivirus that is transmitted mainly by airborne and droplet exposure, although transplacental infection is possible (2). The incubation period of the virus is 14-18 days and an acute to subacute febrile and often fatal disease results (5). The viral infection results in gastrointestinal, respiratory, and CNS manifestations. The initial clinical signs include fever, gastrointestinal signs, and respiratory signs. CNS signs are seen at presentation or after the onset of systemic disease (5). Other clinical signs include hyperkeratosis of the footpads and nose, ptyalism, and enamel hypoplasia (5). The mortality rate is around 50% once contracted

with death occurring in two weeks to three months postinfection (5). The dogs that recover are not carriers of the virus (2).

It is also important to understand the progression of the disease and the body's immune response. One day postinfection, macrophages carry the virus from the upper respiratory tract to regional lymph nodes where replication occurs. By three to six days postinfection, there is viral damage present in the B and T lymphoid cells. One week postinfection, most lymphoid cells are infected and viral shedding is abundant in respiratory exudates. At this time the initial clinical signs of fever and lymphopenia are usually noted. The virus eventually spreads via viremia to the epithelium of the gastrointestinal tract, respiratory tract, and CNS. Canine distemper virus is an immunosuppressive disease and secondary bacterial infections are common and increase the severity of the disease. (2, 5).

The body responds to the viral infection in three different forms. The first form is a strong immune response mounted 14 days postinfection and results in subclinical infections in 25-75% of susceptible dogs. The second form is an intermediate immune response mounted in 9-14 days postinfection that results in a subacute infection. Adequate cellular and humoral immune responses are mounted although viral spread is not prevented. The final form is failure of an immune response 9-14 days postinfection and results in death of the patient in two to four weeks. The virus is usually present in the CNS of most infected patients whether neurologic signs are present or not. Viral spread to the CNS occurs via hematogenous spread to the CSF usually one month postinfection resulting in demyelinating encephalitis, axonal injury, and plasmacytic/lymphocytic infiltrates. (2, 5).

The patient in this case showed respiratory signs three weeks, neurologic signs seven weeks, and was euthanized eight weeks after his second vaccination. Respiratory signs followed

by neurologic signs is a classic presentation for canine distemper virus. Although canine distemper virus was considered in this case, the negative PCR test result from March 21st, 2015 made canine distemper virus lower on the differential diagnosis list. It can now be stated with almost complete confidence that the patient was infected with canine distemper virus when the PCR test was run. The pneumonia and lymphopenia at the time of the test both support the diagnosis as well. As previously mentioned, the PCR test has a high sensitivity indicating that a positive result is highly specific for diagnosing the disease (5). Unfortunately, a negative result is not that effective at ruling out the disease (5). The best time to run the canine distemper virus PCR is within three days of the onset of respiratory signs which is approximately when this puppy was initially tested (1).

In this case, the PCR test was run on a conjunctival swab two days after the onset of respiratory signs. Upon further investigation, it is controversial what the best sample is to submit for early antemortem diagnosis of canine distemper virus infections. In one study, conjunctival swab samples were found to have a higher detection rate in the early diagnosis of canine distemper virus compared to other specimens (7). It is also thought that buffy coat and urine sediment cells are the most sensitive (2). For the best results regardless of disease stage, pooled samples (conjunctival, nasal, urine samples) are recommended by the Animal Health and Diagnostic Center at Cornell (1). In addition, it is possible that the PCR was not able to identify the vaccine strain although evidence is available that proves PCR can distinguish between natural and vaccine virus strains in clinically ill dogs (5). In patients with clinical signs consistent with canine distemper virus that test negative on a PCR test, additional testing is warranted.

The last important point to address is how the patient was infected with canine distemper virus despite proper vaccination. The patient received the Zoetis Vanguard Plus 5 vaccine on an

appropriate schedule until becoming sick three weeks after the second vaccination in the series. This particular vaccine is a modified live virus (MLV) vaccine and contains a canine cell adapted Rockborne like strain (12). MLV vaccines in general provide the best protection against infection and disease but reversion to virulence has been documented (5). A rare fatal encephalitis 7-14 days postinfection occurs in 1 in 10,000 patients vaccinated with the MLV Rockborne strain and in general CNS signs are seen 3-20 days after vaccination with a MLV vaccine (5). Vaccine induced distemper has also been reported in puppies of dams that received a MLV vaccine at whelp or within the first few days postpartum (5).

Diagnosing a natural infection verse a vaccine induced infection is not straight forward. Most of the original diagnostics appear the same including a lymphopenia on bloodwork, possible distemper inclusion bodies present in blood smears, interstitial to alveolar lung patterns on radiographs, and inflammatory CSF changes (5). Detection of a high canine distemper virus antibody titer in the CSF is diagnostic because it is locally produced and therefore not present in vaccinated patients (5). False positives may occur if the CSF is contaminated with blood or if the blood brain barrier is damaged (5). Virus isolation postmortem is difficult in routine cell cultures for natural infections and in vaccine induced cases defective viral replication further decreases success rates at isolation (5). Finally, genetic analysis is able to detect the minor differences in the N gene between natural and vaccine induced infections that have been documented (5). The results of the RNA sequencing being performed by Zoetis will distinguish between a vaccine induced and natural infection.

References:

1. "Animal Health Diagnostic Center." College of Veterinary Medicine Cornell University. 2014. Web. 12 October 2015. <https://ahdc.vet.cornell.edu/>
2. Barr, SC. Bowman, DD. "Canine Distemper." *Canine and Feline Infectious Diseases and Parasitology*. 2nd ed. Ames, IA: Wiley-Blackwell, 2011. 86-91.
3. Cerda-Gonzalez, Sofia. "Neurolocalization" *Block 5b Course Notes.* Cornell U, 2014.
4. Deem, Sharon L., Lucy H. Spelman, Rebecca A. Yates, and Richard J. Montali. "Canine Distemper In Terrestrial Carnivores: A Review." *Journal of Zoo and Wildlife Medicine* 31.4 (2000): 441-51.
5. Greene, Craig E. "Canine Distemper." *Infectious Diseases of the Dog and Cat.* 4th ed. Philadelphia: W.B. Saunders, 2012. 25-42.
6. Gröne, A., Doherr, M. G., and Zurbriggen, A. "Canine distemper virus infection of canine footpad epidermis." *Veterinary Dermatology* 15(3) (2004): 159–167.
7. Kim, Doo, Seok-Yong Jeoung, So-Jeo Ahn, Jong-Hyun Lee, Son-Il Pak, and Hyuk-Moo Kwon. "Comparison of Tissue and Fluid Samples for the Early Detection of Canine Distemper Virus in Experimentally Infected Dogs." *Journal of Veterinary Medical Science J. Vet. Med. Sci.* 68.8 (2006): 877-79. Web. 18 Oct. 2015.
8. Lorenz, Michael D., Joan R. Coater, and Marc Kent. *Handbook of Veterinary Neurology.* 5th edition. St. Louis, MO: Sanders, 2011.
9. Mccormick, A. E. "Canine Distemper in African Cape Hunting Dogs (Lycaon Pictus): Possibly Vaccine Induced." *The Journal of Zoo Animal Medicine* 14.2 (1983): 66.

10. McInnes, Elizabeth F., R. E. J. Burroughs, and N. M. Duncan. "Possible Vaccine-induced Canine Distemper in a South American Bush Dog (Speothos Venaticus)." *Journal of Wildlife Diseases* 28.4 (1992): 614-17.

11. Sutherland-Smith, Meg R., Bruce A. Rideout, Andrea B. Mikolon, Max J.G. Appel, Patrick J. Morris, Amy L. Shima, and Donald J. Janssen. "Vaccine-Induced Canine Distemper in European Mink, Mustela Lutreola." *Journal of Zoo and Wildlife Medicine* 28.3 (1997): 312-18. *American Association of Zoo Veterinarians*.

12. "VANGUARD® PLUS 5." *Zoetis*. 2015. Web. 11 Oct. 2015. < https://www.zoetisus.com/index.aspx>.

13. Wilkes, R. P., Sanchez, E., Riley, M. C., and Kennedy, M. A. "Real-time reverse transcription polymerase chain reaction method for detection of Canine distemper virus modified live vaccine shedding for differentiation from infection with wild-type strains." *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc* 26(1) (2014): 27–34.