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WEST NILE ENCEPHALITIS IN A CAPTIVE FLORIDA SANDHILL CRANE

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Abstract: A 37 year old captive male Florida sandhill crane (*Grus canadensis pratensis*) housed at the International Crane Foundation exhibited abnormal neurologic signs in the fall of 2006. Despite therapy and supportive care, the neurologic signs worsened and the crane was euthanized after 6 days. Antemortem and postmortem serum was positive for flavivirus antibody, and a cloacal swab was positive for West Nile virus (WNV) by reverse transcriptase polymerase chain reaction (RT-PCR). Pectoral muscle atrophy and multifocal myocardial necrosis were observed at necropsy. Histopathologic findings included inflammatory and necrotic lesions in sections of brain, spinal cord, eye, heart, blood vessels, lung, air sac, esophagus, ventriculus, intestine, thyroid, adrenal, kidney, testicle, and feather follicles. A RT-PCR of brain tissue was positive for WNV. Most of the lesions were consistent with what has been described in birds with WNV, but were more severe and broadly distributed. The impact of WNV on captive crane populations has been variable. Currently, 13 of 15 crane species held in captive centers in the U.S. have been seropositive for WNV, but mortality has been limited to sandhill cranes.

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CASE REPORT

West Nile virus (WNV) is a mosquito-borne flavivirus (genus *Flavivirus*, family Flaviviridae) that was discovered in a Ugandan woman in 1937 (Marra et al. 2004). Outbreaks of WNV illness have been recorded periodically in humans in Africa, the Middle East, Asia, and Mediterranean Europe (Zeller and Schuffenecker 2004, Gerhardt 2006) as well as wild birds (Malkinson et al. 2002). In August 1999, the virus was introduced into North America and caused an outbreak of encephalitis in humans, birds, and mammals in and around New York City (Marfin and Gubler 2001). West Nile virus has since spread rapidly throughout North America (Hayes et al. 2005). By 2005, WNV had been detected in all 48 of the contiguous United States and much of Mexico, Canada and the Caribbean (Farfan-Ale et al. 2004, Austin et al. 2004, Godsey et al. 2005, Komar et al. 2005, Beveroth et al. 2006). West Nile virus was first isolated in Wisconsin from two American crows (*Corvus brachyrhynchos*) recovered in Milwaukee County, southeastern Wisconsin, during August 2001 (USGS NWHC, 2001). In the following case report we describe the clinical presentation and progression of WNV-associated disease in a captive 37 year-old male Florida sandhill crane (*Grus canadensis pratensis*) that began exhibiting abnormal neurological signs in September, 2006.

The crane was housed with a similarly aged female in an off-exhibit pen at the International Crane Foundation (ICF) in rural south-central Wisconsin. The pen included a 15 x 18 m outdoor grass-covered yard surrounded by chain-link fencing that was connected to a 4.2 x 4.2 m indoor enclosure. The outdoor portion had 2 inch flight netting across the top and tennis netting along the sides to obscure views and reduce disturbance from neighboring pairs. The crane was exposed to ambient temperature and natural photoperiod. Zeigler crane maintenance diet (Zeigler Brothers, Inc., Gardners, Pa.) and fresh water were provided ad libitum in rubber buckets within the indoor enclosure. The crane's health history was unremarkable except for a chronic malocclusion of the beak that was managed with regular trimming. The crane was serologically negative for flavivirus antibody 2000-2004.

On 14 September 2006 (day 0) the crane was noted to be slightly ataxic. The crane exhibited difficulty balancing, and was quiet and unusually nonaggressive. When approached by handlers the crane walked into a corner of the outdoor pen, gradually lowered itself into sternal recumbency, and minimally resisted manual capture. Abnormal findings on physical examination included low body weight (~80% of seasonal normal) and dull mentation. A complete blood count (CBC) and serum chemistry panel revealed heterophilic leukocytosis with toxic heterophils (15.2 x 10^3 heterophils/µl, 25.3 x 10^3 leukocytes/µl), hypoalbuminemia (1.3 g/dl), hyperglobulinemia (2.3 g/dl), hypophosphatemia (1.1 mg/dl), and elevated creatine phosphokinase (1125 IU/L) (see Olsen et al. 1996 for reference ranges). These findings were consistent with an inflammatory process plus antigenic stimulation in addition to suspected anorexia and muscle wasting or exertion. Primary differential diagnoses included systemic viral (WNV, eastern equine encephalitis [EEE]), and bacterial diseases or toxicological insult such as mycotoxicosis.

The crane was treated immediately with 10 ml/kg of intravenous lactated Ringer's solution (LRS), 15 mg/kg enrofloxacin (Baytril, Bayer HealthCare LLC, Shawnee Mission, Kans.) subcutaneously mixed with LRS, and was locked into the indoor enclosure with its mate.

Due to suspicion of WNV-related disease (rapid onset, clinical presentation, seasonality, and the documented upswing in WNV cases in Wisconsin at the time in the absence of EEE activity), serum was submitted for flavivirus (WNV or St. Louis encephalitis virus) neutralizing antibody testing (Lindsey et al. 1976), and whole blood and a cloacal swab were tested for WNV-specific RNA using reverse transcriptase polymerase chain reaction (RT-PCR) (Marshfield Clinic Research Foundation, Marshfield, Wis.; Lanciotti et al. 2000, Meece et al. 2006). The serum test was positive for flavivirus antibody at a dilution of 1:80. The cloacal swab was weakly positive for WNV, but whole blood was negative.

The severity of the ataxia increased through day 5 of illness. The crane also became progressively weak and unable to achieve a standing position or required the use of walls or its wings to support itself; the crane fell over on several occasions but its posture while recumbent was normal. Treatments included bolus therapy of intravenous (until day 4) or subcutaneous (after day 4) LRS with B vitamins twice daily (Vitamin B Complex, Veterinary Laboratories, Lenexa, Kans.), enrofloxacin subcutaneously twice daily, 90-150 ml of blended enteral feeding formula (8 oz Ensure Plus (Abbott Laboratories, Abbott Park, Ill.), ¼ cup Zeigler crane maintenance pellets, 1 tablespoon Nutrical paste (EVSCO Pharmaceuticals, Buena, N.J.) twice daily, and 1 mg/kg ketoprofen intramuscularly once daily (Ketofen, Fort Dodge Animal Health, Fort Dodge, Ia.).

By day 6 of illness, the crane had developed an intention tremor of the head and neck, was severely ataxic, and had proprioceptive deficits of the wings when recumbent. Due to the progressive neurologic signs and failure to respond to therapy, the crane was euthanized humanely. A final CBC and serum chemistry panel included marked heterophilic leukocytosis (25.06×10^3 heterophils/µl, 38.56×10^3 leukocytes/µl), anemia (packed cell volume = 31%), and an elevated aspartate aminotransferase (695 IU/L). The carcass was submitted to the University of Wisconsin School of Veterinary Medicine (Madison, Wis.) for necropsy.

Gross necropsy findings included moderate pectoral muscle atrophy, chronic, mild to moderate degenerative joint disease of both stifle joints, renal neoplastic foci, and acute, moderate multifocal myocardial necrosis. The renal neoplasia consisted of two raised, smooth, tan nodules that partially extended into the parenchyma of the left kidney consistent with benign adenomas. The myocardial lesions consisted of a 0.9 cm pale focus at the apex of the heart as well as multiple 2-3 mm pale foci scattered throughout the myocardium. Aerobic bacterial cultures of spleen, brain, heart and air sacs yielded no significant growth. Serum samples sent to the National Veterinary Services Laboratory (Ames, Ia.) were positive for neutralizing antibody to WNV at 1:10 dilution, but negative for EEE, western equine encephalitis or Venezuelan equine encephalitis antibody. A RT-PCR test of brain tissue submitted to the Wisconsin Veterinary Diagnostic Laboratory (Madison, Wis.) was positive for WNV.

Microscopically, significant disease affected all organs examined. Subacute inflammatory and/or necrotic lesions were observed in sections of brain, spinal cord, eye, heart, blood vessels, lung, air sac, esophagus, ventriculus, intestine, thyroid gland, adrenal gland, kidney, testicles, and in feather follicles. Most tissues contained mild to moderately severe mixed inflammatory cell infiltrates. Nervous system lesions included meningoencephalitis, gliosis with vasculitis and neuronal satellitosis in the brain, Purkinje cell degeneration and necrosis in the cerebellum, spinal myelitis, neuronal satellitosis and gliosis, and intestinal ganglioneuritis. Moderate regional lymphoid atrophy and lymphocytolysis was observed in the white and red pulp of the spleen. The crane also exhibited chronic lesions consistent with advanced age, including arteriosclerosis of the major blood vessels, spinal lipofuscin, anthracosis in the lungs and air sacs, and testicular atrophy.

DISCUSSION

The majority of the lesions present throughout the tissues were consistent with what has been described for WNV infection in different species of birds (Steele et al. 2000). Vasculitis and necrosis were the most prominent features encountered in multiple tissues including the central nervous, peripheral nervous, cardiovascular, gastrointestinal, urinary, reproductive, ocular, endocrine, integumentary and musculoskeletal systems. The lesions present in the brain and spinal cord were consistent with the neurological signs present in this case.

The lesions we observed were more severe and broadly distributed than unvaccinated adult captive greater sandhill cranes (*G. c. tabida*) infected with 5000 pfu WNV (comparable to one mosquito dose) under laboratory conditions. The disease in these cranes was limited to mild weight loss (~4-8% loss post-infection), and limited histopathological lesions including glial and plasma cell clusters in nervous system tissue and myocarditis in 2 of 5 cranes (G. H. Olsen, USGS Patuxent Wildlife Research Center, Laurel, Md., personal communication). The clinical course and pathological changes observed in the crane from this report were more similar to those described in captive hatch-year Mississippi sandhill cranes (*G. c. pulla*) with acute WNV-related disease (A. Cole, Audubon Center for Research of Endangered Species, New Orleans, La., personal communication). These cranes exhibited weight loss (up to 100g per day) and clinical signs lasting 2-11 days consisting of vague neurologic signs (ataxia and/or weakness) progressing to recumbency. Necropsy findings in 5 of the 7 birds included nonsupperative encephalitis and necrotizing myocarditis.

The impact of WNV on captive crane populations has been

variable since the introduction of the virus in 1999. Five of 6 cranes (83%) from the Bronx Zoo/Wildlife Conservation Park collection were seropositive for WNV antibody following the New York City outbreak in 1999 (Ludwig et al. 2002). None of the cranes showed clinical signs consistent with WNV-related illness. In October 2004, 10% (12/119) of the ICF captive crane flock tested positive for antibodies to flavivirus, suggestive of previous exposure (Hartup 2008). Eight species were represented from the seropositive birds, including North American whooping cranes (*G. americana*), but not sandhill cranes. The seroprevalence within the ICF flock had increased gradually over three years since the spread of the virus to Wisconsin in 2001; however, no clinical disease associated with WNV had been documented in any captive crane at ICF until this case in 2006. At present, 13 of 15 crane species in captivity in the United States have been reported serologically positive for WNV exposure (Travis et al. 2002, USGS NWHC 2005, Hartup 2007), but mortality has been limited to sandhill cranes. A sandhill crane apparently died from WNV infection at a zoo in Bridgeport, Connecticut in fall 1999 (CDC 1999). The one mass mortality involving captive cranes attributable to WNV consisted of loss of seven Mississippi sandhill crane chicks from Louisiana in July 2002 referred to above.

It is unclear what factors may have predisposed this crane to develop WNV-related disease. The developmental stage (or age) of the host is a critical factor in the immune response in birds, along with a variety of nutritional, endocrine and environmental factors (Kollias 1986). In March 2004, the crane's malocclusion and beak overgrowth was suspected to interfere with feeding, leading to a significant loss of weight and body condition. We believe the crane's nutritional status was sound throughout August and September 2006 based on routine beak trimming performed seven weeks prior to the development of clinical signs. The crane was non-reproductive for several years, was not molting, and exhibited no apparent chronic pathological change in the thyroid or adrenal glands that would impact its endocrine function. The pair had a stable territory (pensite) in the ICF breeding colony and was not subjected to extensive management changes through the preceding summer. The crane's clinical pathologic findings gave no indication of organ dysfunction or immune compromise.

We suspect the crane's multiple concurrent chronic health conditions, though minor individually, along with its advanced age, may cumulatively have represented a stressor which impacted the effectiveness of the crane's immune response to WNV infection. The authors of the sandhill crane WNV vaccine study concluded that healthy cranes may not always develop clinical disease from exposure to WNV, but warned that stressed or compromised cranes may develop complications due to WNV infection (G. H. Olsen, USGS Patuxent Wildlife Research Center, Laurel, Md., personal communication). Though not unhealthy, the young Mississippi sandhill cranes described above may have been more susceptible to WNV-related disease and mortality due to their developing immune systems and captive management compared to the lower density adult collection housed nearby that experienced only limited illness and no mortality. In addition, sandhill cranes may possess a species predilection that increases the risk of disease. Sandhill cranes have evolved without WNV in their environment and may exhibit greater susceptibility to its effects compared with species that have co-evolved with the virus (Best et al. 2000).

Lastly, the infective dose of WNV the crane was exposed to remains unknown. In Louisiana, the Mississippi sandhill crane chicks may have been exposed to large quantities of virus during the extensive outbreak involving humans and animals in the New Orleans area during 2002. Though Wisconsin experienced an upsurge in WNV human and animal cases in 2006, the magnitude of viral activity in Sauk County (gauged by wild bird, veterinary and human WNV cases reported to state authorities) where ICF is located appeared similar to 2003-2004. The overall number of cases, however, was significantly less than previous large-scale human and animal outbreaks in more urbanized landscapes such as New York, Chicago, and New Orleans.

ICF houses more than 120 cranes, including 35-40 endangered whooping cranes. While there have been serologically positive whooping cranes at ICF, there has not been a clinical case in this species at the site. At the time of this report, adult captive whooping cranes at ICF are not vaccinated against WNV. Hatch-year whooping cranes destined for release, however, are vaccinated with a killed virus vaccine (West Nile-Innovator, Fort Dodge Animal Health, Fort Dodge, Ia.) due to concerns about crowding and potential immunological susceptibility (Marra et al. 2004). Based on the WNV case in a Florida sandhill crane reported here, re-assessment of the unvaccinated status of the adult whooping crane flock, especially in regards to older, genetically valuable captive breeders, may be warranted.

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