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Authors: Hopf, Cynthia, Bunting, Elizabeth, Clark, Anne, and Childs-Sanford, Sara

Source: Journal of Avian Medicine and Surgery, 36(1) : 85-91

Published By: Association of Avian Veterinarians

URL: https://doi.org/10.1647/20-00112

Journal of Avian Medicine and Surgery 36(1):85–91, 2022

Clinical Report

Survival and Release of 5 American Crows (*Corvus brachyrhynchos*) Naturally Infected With West Nile Virus

Cynthia Hopf, DVM, Elizabeth Bunting, VMD, Anne Clark, PhD, and Sara Childs-Sanford, DVM, MS, Dipl ACZM

Abstract: West Nile virus (WNV) has had a significant effect on avian populations in the United States since being first identified in 1999. Avian species in WNV endemic areas do not suffer the same level of mortality that has been reported in birds within the United States since the virus was first identified in North America. Because of their unique susceptibility, American crows (*Corvus brachyrhynchos*) are often used to monitor the spread and severity of WNV in North America. American crows with WNV infections are received and treated at the Janet L. Swanson Wildlife Hospital (Cornell University, Ithaca, NY, USA) on a regular basis during the summer and fall and have historically had a 100% mortality rate. This report describes WNV-positive American crows that were treated, recovered from the infection, and were subsequently released. The 5 American crows in this case series were tested, when possible, by polymerase chain reaction (PCR) and plaque reduction neutralization on admission and monitored with both PCR and plaque reduction neutralization throughout their rehabilitation process. Four of the 5 birds had a negative PCR test before release, and 1 bird had a "suspect" positive PCR test result before release. One of the crows was confirmed to have survived for at least 2.5 years after release. Viral shedding was documented up to 93 days after initial hospitalization, which is longer than any previous report of WNV shedding in an American crow.

Key words: West Nile virus, WNV, PCR, diagnostic testing, avian, American crow, *Corvus brachyrhynchos*

Clinical Report

Five adult American crows (*Corvus brachyrhynchos*), referred to hereafter as crows 1–5, were presented to the Janet L. Swanson Wildlife Hospital (Cornell University, Ithaca, NY, USA) during the summer and fall of 2017 and 2018. Crows 1 and 2 were presented in September 2017, crow 3 in October 2017, and crows 4 and 5 in August 2018. Crows 1 and 2 were part of 2 family groups of American crows closely monitored by researchers in the local area, whereas crows 3–5 were not. The presenting complaints for all 5 crows included lethargy and a decreased response to human presence. Their physical examinations revealed poor body condition, dehydration, weakness, and neurologic signs that included depressed mentation, ataxia, and tremors. Ophthalmic examinations in all 5 crows were normal. The initial treatment for all of the birds during the first 24 hours of hospitalization included fluid therapy with lactated Ringer's solution (60 mL/kg SC q12h, Hospira Inc, Lake Forest, IL, USA) and meloxicam (1 mg/kg SC q12h, Metacam, Boehringer Ingelheim, St Joseph, MO, USA). Results from complete blood counts and serum biochemistry panels were used to determine treatment needs from that point forward.

On day 2 of hospitalization all crows continued on parenteral lactated Ringer's solution fluid therapy (120 mL/kg per day SC [crows 1, 3, and 4] or IO [crows 2 and 5] administered over a 12-hour period each day), vitamin B complex (10 mg/

From the Janet L. Swanson Wildlife Hospital and the Department of Clinical Sciences, College of Veterinary Medicine (Hopf, Childs-Sanford), and the Department of Population Medicine and Diagnostic Sciences (Bunting), Cornell University, Ithaca, NY 14853, USA; and the Department of Biological Sciences, Binghamton University, Binghamton, NY 13902, USA (Clark).

Corresponding Author: Cynthia Hopf, crh245@cornell.edu

kg SC q12h × 9–16 days; Vitamin B Complex HP, VetOne, Boise, ID, USA), meloxicam (1 mg/kg IO, or SC until able to take PO, q12h × 9–16 days), itraconazole (5 mg/kg PO q24h × 9–16 days; Itrafungol, Elanco, Greenfield, IN, USA), and a broad-spectrum antibiotic. Fluid therapy was continued until the crows were eating well on their own and maintaining or gaining weight. Meloxicam and itraconazole were continued for the entire duration of hospitalization for all 5 crows; therefore, the duration of these medications varied between 9 and 16 days. Crows 1 and 3 were prescribed enrofloxacin (15 mg/kg SC or IO q24h × 10 days, Enroflox, VetOne), crow 2 trimethoprim sulfa (30 mg/kg PO q12h × 14 days, Sulfatrim Pediatric Suspension, Pharmaceutical Associates Inc, Greenville, SC, USA), and crows 4 and 5 amoxicillin trihydrate and clavulanate potassium (125 mg/kg PO q12h × 10 days, Clavacillin Dechra, Overland Park, KS, USA). All crows were treated with antiparasitic medications, including ivermectin (0.4 mg/kg SC once, Noromectin, Norbrook Laboratories, Newry, Northern Ireland), with crows 1–3 and 5 receiving fenbendazole (50 mg/kg PO q24h × 3 days, Panacur, Merck, Kenilworth, NJ, USA) and crow 4 receiving praziquantel (10 mg/kg SC once, Praziquantel, Bimeda, Le Sueur, MN, USA). Antimicrobial selection was made according to varying clinician preferences. Antiparasitic drugs were chosen by historical gastrointestinal parasite findings for crows 1 and 2 and individual fecal analyses for crows 3, 4, and 5.

All crows were housed individually in stainless steel cages (58 cm × 58 cm) with soft cage padding and logs for perches. Their caging was cleaned out twice a day when they were handled for treatments, weighed, and assessed. All crows were handled by gloved veterinary hospital personnel after all other animals had been treated. Food and water bowls were disinfected with 2% Rescue (Animal Health International Inc, Greeley, CO, USA) daily when removed from the cage. The crows received a mixed diet consisting of soaked dog food (Hill's Science Diet Adult 1–6, Topeka, KS, USA), chopped fruits and vegetables, and hard-boiled eggs. Enrichment items, including chopped mice, cereal, and hard dog food kibble, were provided when the birds became more active and were considered to be eating well. Of the 5 crows, individual birds showed preference for different diet items but all were eating normally within 1 week after presentation. Until the crows were eating well on their own, nutritional support was provided with EmerAid Intensive Care Omnivore diet (30 mL/kg PO q8–12h, Emeraid LLC, Lafeber Co, Cornell, IL, USA).

A complete blood count and serum biochemistry panel in crows 3 and 5 showed a mild anemia, heterophilia with bands, reactive lymphocytes, hypernatremia, hyperuricemia, elevated aspartate aminotransferase, elevated creatine kinase, and elevated bile acids compared with reported reference intervals for American crows.[1] Anesthetized, 3-view radiographs were performed on crows 3 and 4, while awake and survey radiographic images were obtained on crow 5. Anesthesia was induced and maintained by facemask with isoflurane. Induction and recovery were uneventful. Views obtained included a ventrodorsal, 30° ventrodorsal, and right or left lateral views. Radiographs for crows 1 and 2 were not obtained because no orthopedic abnormalities were suspected. All radiographic examinations were found to be unremarkable. All 5 crows were tested for West Nile virus (WNV) within 2 days of admission. Choanal swabs and whole blood in ethylenediaminetetraacetic acid were tested for WNV by polymerase chain reaction (PCR), and heparinized plasma was submitted for a plaque reduction neutralization (New York State Animal Health Diagnostic Center, Cornell University, Ithaca, NY, USA) (Table 1). All 5 crows showed clinical improvement and began eating within 1 week of hospitalization. Their neurologic signs and weakness were resolved by 2 weeks, and all 5 crows were discharged to a wildlife rehabilitator for continued care in isolation from other avian patients. Discharge occurred on days 10 (crows 1 and 4), 9 (crow 2), 14 (crow 3), and 16 (crow 5) after presentation.

Crows 1, 2, and 4 were returned for recheck examinations and repeated WNV testing (serology and choanal swab PCR) until the WNV PCR choanal swab was negative (Table 1). Crows 1 and 2 were returned on days 49, 93, and 101 after discharge, and crow 4 was returned on days 39 and 76. Crow 1 was positive by PCR at admission, suspect by day 49, and negative by day 101, and crow 2 was positive at admission and negative by day 49. Similar to crow 2, crow 4 was negative 36 days after admission. Crow 5 was negative at admission but, because of clinical signs consistent with WNV, was tested again 10 days later and had an antibody titer $\geq$320. Crow 5 had a PCR result reported as "suspect" positive before discharge so was housed apart from other birds in rehabilitation for 4 weeks before release. Crow 3 escaped from rehabilitation shortly after discharge, so 1 negative PCR swab was obtained; however, follow-up for

Table 1. Polymerase chain reaction (PCR) and plaque reduction neutralization (PRN) results for 5 American crows after hospitalization and treatment for West Nile virus infection. Cycle threshold (CT) is provided for suspect PCR results. PCR was performed on choanal swabs, and the PRN was performed on heparinized plasma.[a]

Days postpresentation	Crow 1		Crow 2		Crow 3[b]		Crow 4		Crow 5[b]	
	PCR	PRN	PCR	PRN	PCR	PRN	PCR	PRN	PCR	PRN
0–2	POS	≥5120	POS	≥1280	POS	—	POS	2560	NEG	—
8	—	—	—	—	NEG	≥320	—	—	—	—
10	—	—	—	—	—	—	—	—	SUSPECT (CT: 35.52 and 37.99)	≥320
36	—	—	—	—	—	—	NEG	5120	—	—
49	SUSPECT (CT: 33.96)	—	NEG	—	—	—	—	—	—	—
74	—	—	—	—	—	—	—	1280	—	—
93	SUSPECT (CT: 35.52 and 37.57)	—	NEG	—	—	—	—	—	—	—
101	NEG	≥ 320	NEG	≥320	—	—	—	—	—	—

Abbreviations: POS indicates positive (CT < 35 cycles), meaning virus was detected on PCR; NEG, negative (CT > 40 cycles); meaning no virus detected on PCR; SUSPECT (CT = 35–40 cycles), virus was detected at high CT of the PCR.

[a] Dash indicates the test was not performed.

[b] Lost to follow-up.

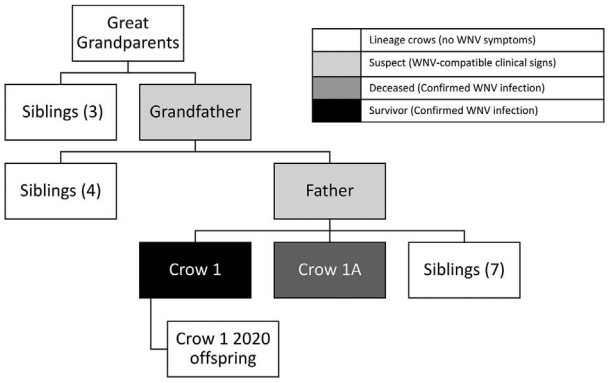

Figure 1. This genealogy tree represents 4 generations in the family of American crow 1. The great grandparents had been observed to show no clinical signs of West Nile virus (WNV), whereas the grandfather and father had signs characteristic of WNV and died during outbreaks in 2009 and 2018, respectively. Crow 1 is the surviving crow from this lineage. It was treated and released after recovering from a WNV infection. Crow 1A is the direct sibling of crow 1. It was observed down on the ground but survived for approximately 2 weeks before the study staff captured it. This crow was hospitalized and treated for WNV but died 4 days later. It was diagnosed with WNV on polymerase chain reaction testing of pooled organ samples postmortem and was diagnosed with concurrent bacterial airsacculitis and pneumonia. As of September 2020, crow 1 was noted to have a surviving clutch of fledglings that was doing well.

this bird was not possible. A prerelease choanal swab to confirm no active shedding was not obtained in this crow.

Physical evaluations of all 5 crows at the time of their recheck examinations were considered normal. Crows 1 and 2 were banded and released back to their home territories after testing was completed. Crow 2 was confirmed to have survived until early spring 2018, and crow 1 was confirmed to be alive and reproductively successful in the summer of 2020. Crows 4 and 5 were not banded and were released back to their home territories later that fall. A detailed lineage is available for crow 1 dating back at least 3 generations (Fig 1).

Discussion

West Nile virus is a zoonotic arthropod-borne flavivirus maintained in an enzootic cycle between mosquitoes and birds, with humans, horses, and most other mammals as dead-end hosts.[2] The American crow is one of many avian species that develop high viremias and shed large amounts of virus in feces, which contributes to maintaining the virus cycle.[2–4] Fecal shedding of virus may contribute to overwintering of the virus and direct bird-to-bird transmission during communal roosting in regions where winters are mild.[3,5] West Nile virus has had a tremendous adverse effect on a variety of avian populations in the United States since it was first identified in New York City in 1999.[6–12] American crows are severely affected by

WNV, and population declines highlight the significance of this disease for the crow population.[6,10] A decline of up to 45% in the regional American crow population immediately after the identification of WNV was documented in breeding bird surveys.[10] Since the initial spread of WNV, the severity of WNV outbreaks across North America has fluctuated because of a multitude of vector, host, and environmental factors that are not completely understood.[9,13,14]

American crows are one of the most sensitive avian species to WNV, with a mortality rate of 100% documented in both experimentally and naturally infected crows.[2,12,15] This high mortality rate is not universal for all corvids. American crows were found to have 100% mortality when inoculated with the NY99 WNV strain, whereas fish crows (*Corvus ossifragus*) were found to have a mortality rate of only 55%–70%.[2,16] Consequently, because of their high sensitivity to WNV, American crows are often used as a sentinel species in public health efforts to monitor for the presence of virus activity in the environment and the risk of potential outbreaks in humans.[5,8,9,12,13,17–19] The Swanson Wildlife Hospital has contributed to annual WNV surveillance efforts by the New York State Department of Environmental Conservation by testing suspect WNV-positive crows along with other corvids and raptors from 2013 to 2020. The purpose of this WNV case series was to describe the presentation, diagnostic testing, and treatment of the only 5 hospitalized crows that survived WNV during this time period. Moreover, this clinical report includes the documentation of shedding and antibody titers in 3 of the crows, and the genealogy of 1 of the crows. The survival of these 5 American crows is notable given the historically high mortality seen with this virus in this species.

WNV has dramatic pathophysiologic effects in American crows that drive a rapid clinical course ending in death. Severe dehydration, acid-base and electrolyte imbalances, cellular injury, and multiorgan inflammation and necrosis described in experimental infection of American crows likely contribute to their poor clinical outcome. Historically, only supportive fluid and nutrition strategies have been recommended for treating WNV-positive crows when presented with clinical illness.[15] The crows in this case series exhibited clinical signs and biochemical changes consistent with previous reports in this species.[15,16,20,21] The intensive supportive care provided to the crows in this case series included IO or SC fluid therapy, anti-inflammatory medication, B vitamin supplementation, and prophylactic antiparasitic medication. Additionally, because of the propensity of American crows to develop secondary bacterial and fungal infections during WNV infection, antibiotic and antifungal therapies were also administered.[15] Because crows 1 and 2 were part of a closely monitored population, their clinical illness may have been recognized at an earlier stage than would typically occur. Administration of fluid therapy and other treatments early in the course of infection may have contributed to the successful outcome in these 2 cases, although the stage of infection was unknown. Nevertheless, the treatment plan employed in these 5 crows is similar to those used in previous and subsequent years at Swanson Wildlife Hospital, as well as at other wildlife hospitals; thus, other contributing factors to the successful recoveries must be considered.[22] An additional 32 American crows were presented to the Swanson Wildlife Hospital during this time period, and 25 were confirmed WNV-positive with PCR. Only the 5 discussed in this case series survived to release.

One possible reason for the survival of the 5 American crows described in this report may be adaptation of American crows to the virus, which has been demonstrated at the immunologic level in some avian species. In WNV-endemic areas where birds have lived with the virus for centuries, the native birds, including *Corvus* species, have developed increased resistance to the virus and the mortality rate is low.[23] House sparrows (*Passer domesticus*), which develop high viremias similar to American crows, have been shown to be adapting to WNV over time.[7] House sparrows experimentally exposed to the WNV NY99 strain developed lower viremias in 2013 than they did in the early 2000s. Conversely, those collected from 2012 to 2013 continued to develop a higher viremia to newer strains of WNV (WN02 and SW03).[7] Furthermore, when a more recent and more virulent viral genotype was held constant during experimental infection, house sparrows developed lower viremias over time. This study demonstrated increased resistance can occur as the avian hosts coexist with the virus.[24] The survival of the crows presented in this case series may be evidence that the American crows in this population are beginning to adapt to WNV, despite a relatively high mortality rate. Reed et al[19] suggested that American crows were beginning to adapt to WNV in New Jersey, USA, only a few years after it arrived. However, despite surveillance at Swanson Wildlife Hospital, until now no clinical cases of survival have been reported in any WNV-positive Ameri-

can crows. Two of the crows (crows 1 and 2) originated from monitored family groups of American crows, and observations and data suggest they lived in areas with regular WNV outbreaks since 2002.[6] The paternal grandfather of crow 1 disappeared during a WNV outbreak in 2012, and this crow's father also disappeared in 2018 after being observed with clinical signs consistent with WNV. The full sibling of crow 1 (denoted crow 1A in Fig 1) was captured in 2018 after 2 weeks of being observed in the wild with clinical signs of WNV and died despite hospitalization and treatment. Both WNV infection and bacterial airsacculitis and pneumonia were confirmed postmortem. This history suggests that the family of crow 1 was present during more than 1 WNV outbreak, and genetic resistance to the virus may have occurred over time, with crow 1's sibling surviving for 2 weeks in the wild with WNV infection before capture and death, and crow 1 surviving treatment and successful release.

Variation in the virulence of the circulating viral strain is another possible reason for survival in these cases. The high avian mortality rate after the identification of WNV in North America was suspected to be due to both immunologically naïve avian populations and the high virulence of the introduced strain (NY99).[20] West Nile virus has continued to evolve in North America, resulting in a variety of strains or variants, including WN02 and SW03, and the extinction of NY99.[25,26] Indeed, certain genetic changes have been demonstrated to alter the virulence of the virus in avian hosts, including American crows.[17,18] New WNV strains, such as NY01, NY07, and NY10, have increased in prevalence and displaced historic strains in New York State.[25] Additionally, strain isolates from avian tissues in New York demonstrated greater diversity than isolates from mosquitoes, indicating that birds may offer important information regarding evolution and emergence of new WNV strains.[25] The WNV strain resulting in clinical disease in the crows in this case series was not determined because of a lack of sufficient sample volume for virus isolation. However, surviving crows like the 5 reported here may be an important source of viral genetic material to help better understand the effects of an evolving WNV regarding avian morbidity and mortality.

Numerous avian species develop neutralizing antibodies to WNV, which can be protective against subsequent infection for many years and can also be passed on to offspring to provide maternal antibody protection in chicks.[27–29] Antibody persistence and decay studies in American crows, fish crows, and house sparrows reflect that the prevalence and duration of antibody titers varies significantly between species.[11,28] Detectable antibodies were observed for 12 months in captive fish crows, a corvid species with a lower mortality rate than American crows.[11,16] Historically, experimental and natural infections with NY99 have caused 100% mortality in American crows. Because of the acutely fatal nature of WNV infection in American crows, an antibody response to natural infection has not been well documented or described.[16,20] In the 5 crows in this case series, WNV antibody titers were quantified by a plaque reduction neutralization test. All 5 crows mounted an antibody response of >320 (Table 1). Crows 1 and 2 continued to maintain an antibody titer ≥ 320 until the PCR choanal swabs were negative, which was just before release at 101 days after hospitalization. This is the first serial collection of antibody titers occurring after natural infection of WNV in American crows. The measurement of WNV antibody titers in American crows presented to wildlife hospitals and rehabilitation centers may be a way to monitor for seropositive crows that are surviving WNV.

To detect shedding of WNV in these individual crows, choanal swabs were tested for WNV nucleic acid by PCR. Choanal swabs have been determined to be the best site for sampling WNV nucleic acid in corvids.[30] Choanal swabs have been used to monitor WNV shedding in American crows in an experimental infection study and as a method of surveillance of American crow carcasses during mortality events.[4,16] Komar et al[16] detected oral shedding in American crows up to their death 5 days postinfection and in fish crows up to day 9. In experimental studies, oral viral shedding in American crows has not been documented for longer than 5 days because of experimental design, rapid mortality of the study subjects, or both.[16,21] During the follow-up evaluation of the crows described in this case study, choanal swabs were used to measure shedding at intervals before release. Because of long intervals between testing, we cannot verify when the crows stopped shedding between their initial positive PCR and their negative PCR. A suspect positive result indicated a high cycle threshold of between 35 and 40 cycles for the WNV PCR. The cutoff for a positive result with this WNV PCR was a cycle threshold of 35 cycles, which is the upper limit of detection for this assay. In this case, the suspect positive result indicated the presence of a small number of WNV particles in the sample. The cycle thresholds for suspect positive cases are provided in Table 1.

Given these results, crow 1 stopped shedding between days 93 and 101, when the test results switched from suspect to negative. This may also explain how crow 5's test switched from negative to suspect, indicating shedding of a small amount of WNV during the course of infection. This 93-day period is a longer interval of shedding than previously reported for American crows. More frequent testing would be helpful in future cases to track how long birds shed WNV.

In American crows experimentally infected with the NY99 WNV strain, viremia occurred as early as 1 day postinfection and persisted until euthanatization or death on day 4 or 5, with virus detected in almost all organ systems.[21] Gross pathologic findings included splenomegaly, free coelomic fluid, and pale tan livers, and histopathologic changes included multifocal hepatocellular degeneration, mild renal tubular necrosis, splenic lymphoid depletion and necrosis, myocardial degeneration, and inflammation in many organs, including the gastrointestinal tract, cerebrum, and vasculature.[15] The immunologic and physiologic response characteristics of American crows may be contributing factors to their high morbidity and mortality, although there has been little investigation in this area. Chronic changes and persistence of virus in affected organs, as has been demonstrated in numerous raptor species, have not been described in American crows because of acute mortality.[15] Active or permanent pathologic changes in the 5 crows in this case series cannot be ruled out; however, crow 1 has survived several winters and reared offspring, which are good measures of success for a wild bird. Long-term follow-up postrelease with postmortem examination would have been necessary to confirm the presence or absence of chronic pathologic effects of WNV infection.

This case series documents the survival of American crows after treatment for clinical WNV disease, and all cases were rehabilitated and released. Treatment was initiated in a similar manner to previously treated cases of WNV in American crows presented to the hospital. Viral shedding and antibody persistence were monitored to varying degrees in these patients, and we found a WNV antibody persistence for at least 93 days in American crows. One crow is part of a closely monitored group of individuals and has continued to thrive in the wild 2.5 years after release. Other factors possibly influenced the survival of these crows, such as proximity to houses or public places facilitating earlier rescue, proximity to better food sources, stronger family groups providing for better nutrition despite their illness, or even more favorable weather conditions during the time period the 5 birds became ill. These factors were not investigated in this case series but could certainly have affected the survival of some of these birds. The results of this case series suggest that the relationship between American crows and WNV strains in the United States is continuing to evolve. Understanding more about how the WNV virus interacts with birds and the coevolutionary changes occurring in avian hosts, vectors, and the virus can lead to new information that may aid in understanding outbreaks in both birds and humans.

Acknowledgments: We thank the New York State Department of Environmental Conservation's Wildlife Health Program for funding the diagnostic testing used to assess the health of the crows.

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