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Source: Journal of Wildlife Diseases, 43(2) : 206-213

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-43.2.206>

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RAPTOR MORTALITY DUE TO WEST NILE VIRUS IN THE UNITED STATES, 2002

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ABSTRACT: West Nile virus (WNV) has affected many thousands of birds since it was first detected in North America in 1999, but the overall impact on wild bird populations is unknown. In mid-August 2002, wildlife rehabilitators and local wildlife officials from multiple states began reporting increasing numbers of sick and dying raptors, mostly red-tailed hawks (*Buteo jamaicensis*) and great horned owls (*Bubo virginianus*). Commonly reported clinical signs were nonspecific and included emaciation, lethargy, weakness, inability to perch, fly or stand, and nonresponse to danger. Raptor carcasses from 12 states were received, and diagnostic evaluation of 56 raptors implicated WNV infection in 40 (71%) of these cases. Histologically, nonsuppurative encephalitis and myocarditis were the salient lesions (79% and 61%, respectively). Other causes of death included lead poisoning, trauma, aspergillosis, and *Salmonella* spp. and *Clostridium* spp. infections. The reason(s) for the reported increase in raptor mortality due to WNV in 2002 compared with the previous WNV seasons is unclear, and a better understanding of the epizootiology and pathogenesis of the virus in raptor populations is needed.

Key words: Avian disease, avian viral infection, histopathology, raptor mortality, West Nile virus.

INTRODUCTION

West Nile Virus (WNV) has quickly spread across the North American continent and into Latin America and a number of Caribbean islands (Hayes et al., 2005). Although WNV is maintained through a bird-mosquito transmission cycle, WNV epidemics in the Eastern Hemisphere primarily have affected human and equine cases. A characteristic of the North American epidemic is overt avian mortality. Since 1999, hundreds of thousands of dead birds have been reported in the United States, and tens of thousands have tested positive for WNV (Centers for Disease Control and Prevention [CDC]/ArboNet contributors, unpubl. data). Because of inconsistencies in surveillance activities both geographically and temporally, it is impossible to accurately assess the impact of WNV on wild bird populations. Although corvids (crows, jays, and ravens) have been the focus of many surveillance programs, raptors often have been included in surveillance programs because of their high visibility; however,

relatively few tested positive from 1999 to 2001 (CDC/ArboNet contributors and US Geological Survey National Wildlife Health Center [NWHC], unpubl. data).

Beginning in August 2002, raptor rehabilitators and wildlife biologists reported an increase in reports and admissions of sick and dying raptors to the US Geological Survey NWHC. Red-tailed hawks (*Buteo jamaicensis*) and great horned owls (*Bubo virginianus*) were most frequently reported and displayed nonspecific clinical signs. Because this increased morbidity and mortality in raptors was occurring in areas of confirmed WNV activity, WNV was suspected as the cause; in response, an investigation was initiated in August 2002.

MATERIALS AND METHODS

The NWHC solicited submissions of raptor carcasses from states reporting increased raptor mortality from mid-August through October 2002. Submissions consisted of frozen or chilled birds that had been dead for less than 24 hr or that had been in the care of a rehabilitation facility for less than 24 hr

before euthanasia. Birds solicited from wildlife rehabilitators had received no treatment other than food and fluids. Each carcass underwent gross and microscopic evaluation and routine testing for viral, bacterial and fungal infection(s), exposure to lead, and exposure to organophosphate or carbamate compounds. Tissues collected for histopathologic examination included brain, heart, liver, kidney, and spleen. Virus isolation from tissues (usually brain and kidney/spleen, occasionally heart and/or other tissue samples) was done on Vero cells (Docherty and Slota, 1988), and virus isolates were confirmed as WNV by reverse transcriptase-polymerase chain reaction (RT-PCR; Lanciotti et al., 2000). Liver samples were collected routinely for microbiologic culture; other samples were collected based on gross examination. Tissues samples were streaked onto blood agar and eosin methylene blue agar for bacterial culture and embedded in Sabouraud dextrose medium for fungal culture (Baron and Finegold, 1990). Liver tissue was tested for lead by atomic absorption spectrophotometry, and brain cholinesterase activity (Hill and Fleming, 1982) was measured to detect organophosphate and carbamate exposure.

Cases were initially confirmed as positive for WNV infection if virus had been isolated and histopathologic changes consistent with WNV infection were noted. Suspect WNV cases were classified into two groups: 1) those from which WNV had been isolated from tissues but had insufficient histopathologic findings to support viral infection as contributing to death and 2) those from which WNV had not been isolated but had histopathologic lesions suggestive of viral infection. For suspect cases from which quality tissue samples were available, immunohistochemistry (IHC) was performed to visualize flavivirus antigen. For IHC, paraffin-embedded tissue sections were sent to the Department of Pathology (College of Veterinary Medicine, The University of Georgia, Athens, Georgia, USA) where they were deparaffinized and hydrated via xylene and graded alcohol washes. Antigen retrieval was done with protease III (Ventana, Tucson, Arizona, USA) for 3 min at room temperature. Slides were blocked with Universal Blocking Reagent (Biogenex, San Ramon, California, USA) for 8 min at room temperature. The remainder of the IHC procedure was done on a Ventana automated stainer by using rabbit polyclonal WNV antibody (1:500 dilution, BioReliance, Rockville, Maryland, USA) followed by alkaline phosphatase reagents; fast red was used as the chromagen and hematoxylin as the counter-

stain. Suspect cases were considered confirmed if IHC results supported WNV infection.

A comparison of the ability to isolate WNV from the different tissues (brain, kidney/spleen, heart) was performed using McNemar's test. Associations of WNV suspect and confirmed cases with species, gender, age, clinical presentation, and histopathologic changes were evaluated by logistic regression with SAS version 8 (SAS Institute, Cary, North Carolina, USA). Statistical analyses were evaluated at $\alpha=0.05$.

RESULTS

Reports of increased raptor mortality were received from 12 states, including Iowa, Illinois, Indiana, Kentucky, Maryland, Michigan, Missouri, Nebraska, Ohio, Pennsylvania, Virginia, and Wisconsin. Fifty-six raptor carcasses representing 13 species were submitted from these states to the NWHC from mid-August through October 2002 (Table 1). Submissions included more females ($n=37$) than males ($n=19$) and included 32 adults (57%), 11 subadults (20%), 11 juveniles (20%), and two birds of undetermined age (3%). Clinical presentations of these cases are given in Table 2.

Forty-five (80%) of the examined birds were emaciated or in poor body condition (no observable body fat with varying levels of muscular atrophy) and 11 (20%) birds were in fair to excellent condition (fair to abundant body fat). Other gross findings at necropsy included trauma ($n=6$), mycotic pneumonia ($n=4$), anemia ($n=2$), gastric ulcer ($n=1$), myocarditis ($n=1$), bilateral conjunctivitis ($n=1$), stomatitis ($n=1$), nematode infection ($n=1$), typhlitis ($n=1$), and fibrinous peritonitis ($n=1$).

None of the birds had depressed cephalic cholinesterase activity, indicating that they had not been exposed to carbamate or organophosphate compounds. Lead was detected in the liver tissue of two bald eagles (1.02 ppm and 16.80 ppm). Parasitic infections were detected in seven carcasses and included minimal to mild sarcosporidiosis ($n=4$),

TABLE 1. Raptors submitted to the National Wildlife Health Center (NWHC) and tested for West Nile virus (WNV) during the 2002 WNV season. WNV test results are based on virus isolation from selected tissues and confirmation of virus isolates by reverse transcriptase polymerase chain reaction.

Common name	Scientific name	No. submitted (% positive)
Red-tailed hawk	<i>Buteo jamaicensis</i>	20 (50)
Great horned owl	<i>Bubo virginianus</i>	16 (56)
Sharp-shinned hawk	<i>Accipiter striatus</i>	5 (40)
Peregrine falcon	<i>Falco peregrinus</i>	2 (0)
Bald eagle	<i>Haliaeetus leucocephalus</i>	3 (67)
Cooper's hawk	<i>Accipiter cooperii</i>	2 (0)
Barred owl	<i>Strix varia</i>	2 (0)
Prairie falcon	<i>Falco mexicanus</i>	1 (100)
Northern goshawk	<i>Accipiter gentilis</i>	1 (100)
Merlin	<i>Falco columbarius</i>	1 (0)
Rough-legged hawk	<i>Buteo lagopus</i>	1 (0)
Eastern screech owl	<i>Megascops asio</i>	1 (0)
Turkey vulture	<i>Cathartes aura</i>	1 (0)

renal coccidiosis ($n=1$), and unspecified gastrointestinal helminths ($n=4$). Fungal infections (aspergillosis) were present in five raptors (9%), and bacterial infections (*Salmonella* spp. and *Clostridium* spp.) were detected in three raptors (5%). West Nile virus was isolated from 25 (45%) of

the raptors (Tables 1, 3). There was no significant difference in WNV isolation rates from the different tissues tested (brain vs. kidney/spleen pool vs. heart; $P>0.50$).

Of the 56 birds received, histopathologic examination of tissues could be per-

TABLE 2. Clinical presentation of raptor cases included in this investigation. Information on the clinical presentation (found alive vs. dead) was not provided for one red-tailed hawk from which West Nile virus (WNV) had been isolated.

	No. overall (% of total)	No. suspect/confirmed WNV cases (% of total)
No. of cases	56	40
Found dead	10 (18)	5 (13)
WNV isolated	1 (10)	2 (40)
Found alive		
Died within 24 hr	35 (76)	26
WNV isolated	16 (46)	15 (58)
Euthanized within 24 hr	11 (24)	9
WNV isolated	7 (64)	7 (78)
Clinical signs		
No. cases with information	38	29
Emaciation/very thin	14 (37)	13 (45)
Inability to walk, perch, fly	14 (37)	7 (24)
Lethargy	11 (29)	7 (24)
Head tremors or bobbing	9 (24)	4 (14)
Weakness	8 (21)	8 (28)
Decreased or no response to danger	4 (10.5)	4 (13.8)
Blindness or other neurologic abnormalities of eyes	4 (10.5)	4 (13.8)
Leg paresis or paralysis	4 (10.5)	2 (6.9)
Anorexia or inability to swallow	3 (7.9)	2 (6.9)

TABLE 3. Comparison of virus isolation (VI) and immunohistochemistry (IHC) results among tissues tested among the 40 raptors considered suspect or confirmed West Nile virus cases.

	Brain (% of total)	Kidney/ spleen (% of total)	Heart (% of total)
Virus isolation			
Total no. tested	17	24	8
No. positive	10 (59)	16 (67)	4 (50)
Immunohistochemistry			
Total no. tested	8	8	12
No. positive	0	3 (38)	4 (33)
Total no. both VI and IHC performed ^a	7	7	5
VI +, IHC +	1 (14)	0	1 (20)
VI +, IHC -	1 (14)	0	1 (20)
VI -, IHC +	0	4 (57)	1 (20)

^a + = virus detected; - = virus not detected.

formed on only 47 due to severe autolysis or freeze artifact. Histopathologic findings of viral encephalitis and/or myocarditis confirmed WNV as the cause of death in 13 of 25 virus isolation-positive birds. The bald eagle with the liver lead level of 1.02 ppm also had WNV isolated from its tissues and had mild multifocal encephalitis, supporting viral infection (not lead poisoning) as the cause of death. Among the WNV-positive birds were two red-tailed hawks in which illness could be attributed to aspergillosis or *Clostridium* spp. infection. West Nile virus was not isolated from the tissues of 31 raptors, although 17 of these raptors had histopathologic lesions suggestive of viral encephalitis and/or myocarditis. Among these birds, were two red-tailed hawks that were determined to have died from aspergillosis and one great horned owl from which Saint Louis encephalitis (SLE) virus was isolated.

Tissues were selected from 24 birds for IHC testing, and on the basis of virus isolation results, 10 of these birds were positive for WNV and 14 birds were negative. According to the initial WNV

case classification, five birds were confirmed, 16 birds were suspect, and three birds were negative. Flavivirus antigen was detected in all five raptors that classified as WNV cases based on virus isolation and histopathology. Antigen was not detected in two raptors whose deaths were attributed to other causes. Among 16 suspect cases, flavivirus antigen was detected in the tissues of three (60%) of the virus-positive suspect cases and six (55%) of the virus-negative suspect cases. Virus isolation and IHC results on the same tissues from confirmed and suspect cases did not necessarily correspond with each other (Table 3).

On the basis of virus isolation, histopathology, and IHC, 40 raptors were considered confirmed or suspect WNV cases. Among the confirmed or suspect cases, histopathologic examination of tissues could be performed on only 35 birds. The most common histopathologic findings among the 35 raptors were nonsuppurative encephalitis and myocarditis that varied in severity and location within the tissues (Table 4). When comparing virus isolation results and histopathologic findings, WNV was isolated from brain tissue of only four of 21 (19%) raptors with encephalitis, from kidney/spleen tissue pools of four of seven (57%) raptors with histopathologic changes in spleen and/or kidney, and from the heart tissues of four of nine (44.4%) raptors with myocardial necrosis and/or inflammation. Conversely, WNV was isolated from the brains of two raptors without encephalitis or other brain tissue changes as well as from kidney/spleen tissue pools from 10 raptors without histopathologic changes in either the kidneys or spleen.

Infection with WNV was not associated with age, gender, species, clinical presentation, or histopathology (univariate analysis, $P>0.10$; data not shown). Results were consistent even when controlling for age and gender among the species ($P>0.05$; data not shown).

For the 16 WNV-negative birds, cause

TABLE 4. Histopathologic changes found in 38 confirmed or suspected West Nile virus (WNV) cases. These cases include 21 raptors from which WNV was isolated and histopathology supported WNV as cause of death and 17 raptors from which virus was not isolated but histopathologic lesions were supportive of viral infection.

Species	No. of cases	Encephalitis	Myocarditis	Myelitis	Hepatitis
Red-tailed hawk ^a	16	13	9	4	2
Great horned owl	11	8	8	2	3
Sharp-shinned hawk	4	3	3	0	0
Bald eagle	2	2	1	1	1
Peregrine falcon	1	1	0	0	0
Cooper's hawk	1	1	1	0	0
Goshawk	1	0	1	0	0
Barred owl	1	1	0	0	1
Prairie falcon	1	1	0	0	0
Total	38	30	23	7	7

^a Autolysis precluded histopathologic evaluation of two red-tailed hawks from which WNV was isolated; therefore, data from these birds are not included among the cases.

of death was related to trauma ($n=5$); aspergillosis ($n=3$); concurrent *Salmonella* spp., *Aspergillus* spp., *Sarcocystis* spp., and unspecified helminth infections ($n=1$); salmonellosis ($n=1$); lead poisoning ($n=1$); and gastric ulcer ($n=1$). The cause of death in the remaining four birds was undetermined due to extensive tissue autolysis. In the five WNV-negative birds that died from trauma, histopathologic lesions suggestive of viral infection also were not observed.

DISCUSSION

This investigation was initiated to determine the cause of the increased reports of raptor morbidity and mortality during 2002. Although the number of sick raptors reported by wildlife rehabilitators and wildlife biologists during 2002 seemed to have increased, hard evidence supporting a true increase of raptor mortality in these regions during this time is lacking. The presenting clinical signs of the raptors were nonspecific, and they could have resulted from a number of avian diseases. Our investigation found that WNV likely played a role in the deaths of 40 of the 56 raptors evaluated. However, a clear picture to characterize WNV infection in the raptor species evaluated could not be developed.

Among the 40 cases, virus isolation, IHC, and histopathologic results were inconsistent. There were also cases from which WNV had been isolated, but another cause of death was determined. Rapid progression of neuroinvasion and central nervous system involvement may explain cases with positive virus isolation and/or IHC results and mild or no tissue changes; this scenario has been reported with other viral encephalitides (Chambers and Diamond, 2003). When considering the differences in virus isolation and IHC results, it is possible that the length of time that tissue samples were in formalin may have affected IHC results (Leong and Gilham, 1989; van Alstine et al., 2002). However, positive IHC results were received from tissues of three raptors that had been in formalin for at least 18 days and six of the 11 birds that were negative by IHC had been in formalin for less than 6 days before being processed for histopathology slides. Although substantiating evidence is lacking, cases that were WNV negative by virus isolation but had histopathologic change consistent with infection may represent recovering or long-term infected individuals where virus was below detectable limits for isolation; this situation could be enhanced if histopathologic changes were immune mediated. Controlled experi-

mental infection studies would help to more clearly define the pathogenesis of WNV in these species.

The clinical presentation and findings of encephalitis and myocarditis as well as some of the other histopathologic lesions due to WNV infection are similar to those reported in naturally infected raptors during the same and subsequent WNV seasons (Fitzgerald et al., 2003; Wünschmann et al., 2004, 2005; Nemeth et al., 2006b) and in experimentally infected raptors (Nemeth et al., 2006a, b). Although fatal infection most commonly involved the brain and heart, we did not find distinct differences in pathology among the species submitted; severity of lesions varied and locations of lesions varied among and within tissues. Small sample sizes among most of the species submitted for the investigation, however, likely limited our ability to detect potential species-related differences as reported by Wünschmann et al. (2005).

A great horned owl with mild cardiac lesions was positive for SLE by virus isolation. Heart tissue from this bird was also flavivirus positive; the IHC method used in this study is flavivirus rather than WNV specific (Howerth, pers. comm.). Because the samples in this study represented clinical submissions, it is possible but unlikely that other IHC-positive/virus isolation-negative birds were erroneously considered as a WNV-confirmed or WNV suspect case. Although the isolation of SLE virus, IHC-positive results, and the mild cardiac lesions found in this owl suggest that SLE may have played a role in its death, SLE virus infections are generally not associated with avian morbidity or mortality (Reisen, 2003).

Although the investigation was able to show that WNV, not another major wildlife disease event, played a role in the cases seen, there are some limitations to this investigation. The states from which the NWHC received raptor carcasses were no longer testing birds in the geographic areas where the raptors were found. Anecdotal

reports from wildlife rehabilitators of sick raptors also were received from several other states that were still conducting avian WNV testing; testing on these raptors, however, may have been performed as part of the state surveillance program, and diagnostic findings of these raptors were not available to NWHC. In addition, red-tailed hawks and great horned owls were the most represented among the cases received. At the time NWHC was contacted regarding the raptor cases, some wildlife rehabilitators and biologists had already identified red-tailed hawks and great horned owls as those raptors primarily affected; this early identification may have biased which species were submitted to NWHC. It is possible that raptor mortality associated with WNV occurred in other states and affected other species that were not adequately represented in this investigation.

The reason(s) for the reported increase in WNV raptor cases in 2002 compared with previous WNV seasons is still unclear. It is possible that increased awareness of WNV may have led to increased reporting; cases were being reported primarily in the states experiencing high rates of WNV infection. However, increased reports of raptor morbidity or mortality were not received in previous years from others experiencing WNV outbreaks. The possibility of consumption of infected prey leading to infection of raptors was suggested previously (Garmendia et al., 2000), and laboratory studies suggest that virus can be transmitted to great horned owls, crows, and other passerines via oral ingestion of experimentally infected prey and mosquitoes (Komar et al., 2003). The importance of this route of infection in the natural environment is unknown, but it may have contributed to the increase in infected raptors as a result of becoming debilitated by WNV infection and more prone to predation. The role of small mammal populations in virus maintenance in the environment is unknown. Recent studies

of wild mammals support that some mammals may be exposed to WNV at high rates (Root et al., 2005) and may potentially serve as reservoirs (Root et al., 2006). If WNV titers in wild rodents are sufficient, consumption of rodents by raptors also may have contributed to the increased WNV infection in 2002. The direct impacts of WNV on raptor populations, along with the indirect impacts on other wildlife populations, such as prey (e.g., rodent) populations, are unknown.

Known avian diseases, other than WNV, could cause similar nonspecific signs in raptors, such as starvation, trauma, and lead poisoning. In addition, there may be other as yet not identified diseases in wildlife populations that adversely affect raptors. The emergence of WNV in North America and a related flavivirus, Usutu virus, in Europe, and the detection of new rhabdoviruses in birds emphasize the importance for diagnostic evaluation of wildlife mortality events to detect the emergence of "new" diseases (Travassos da Rosa et al., 2002; Weissenböck et al., 2002).

ACKNOWLEDGMENTS

The authors thank the many wildlife rehabilitators and state public health and wildlife officials for invaluable assistance in submitting cases to the NWHC for the investigation. In addition, the authors acknowledge the assistance of the many NWHC diagnostic staff members; E. Hofmeister confirmed the SLE isolate by RT-PCR. The immunohistochemistry staining of tissues was performed at the Department of Pathology (School of Veterinary Medicine, The University of Georgia) by A. Ellis and read by E. Howerth.

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Received for publication 23 August 2005.