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WEST NILE VIRUS DETECTION IN THE ORGANS OF NATURALLY INFECTED BLUE JAYS (*CYANOCITTA CRISTATA*)

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ABSTRACT: Blue jays (*Cyanocitta cristata*) are an effective indicator species for West Nile virus (WNV) and may be regionally important in surveillance efforts. The sites of WNV replication and sensitivity of virus detection techniques are undefined for blue jays. The objectives of this study were to describe the gross and microscopic pathology associated with natural WNV infection in blue jays, as well as determine the most appropriate tissues to be used for virus isolation, reverse transcription–nested polymerase chain reaction, and immunohistochemistry (IHC) techniques. Blue jays were collected in Georgia, USA, between May and September 2001. Initial screening by virus isolation indicated that 36 of 59 blue jays chosen for evaluation were WNV positive. From this group, 20 positive and five negative birds were chosen to compare virus detection techniques. Six positive and five negative birds were selected for histopathology examination. Splenomegaly and poor body condition were the most consistent gross findings among positive birds. The most consistent histopathologic findings in the tissues of WNV-positive blue jays were mononuclear leukocytosis and epicarditis/myocarditis. Brain, heart, and lung had the highest viral titers, and WNV antigen was most often detected by IHC in heart, kidney, liver, and lung. Reverse transcription–nested polymerase chain reaction proved to be the most sensitive diagnostic test applied in this study irrespective of the tissue type. Brain tissue could be used effectively for both virus isolation and RT-nPCR, and this tissue is simple to remove and process. The success of IHC is highly dependent on tissue selection, and the use of multiple tissues including heart, kidney, liver, or lung is recommended.

Key words: Avian, immunohistochemistry, pathology, reverse transcription–nested polymerase chain reaction, virus isolation, West Nile virus.

INTRODUCTION

Since its initial discovery in the United States during the summer of 1999, West Nile virus (WNV) has caused extensive bird mortality across North America. American crows (*Corvus brachyrhynchos*) are particularly susceptible to WNV, with mortality rates of 100% reported from experimental infection studies (McLean et al., 2001; Komar et al., 2003). Other corvids, such as black-billed magpies (*Pica hudsonia*), blue jays (*Cyanocitta cristata*), and fish crows (*Corvus ossifragus*), are also susceptible to disease caused by WNV (Komar, 2000; Steele et al., 2000). For this reason, corvids have been used extensively for mortality-based WNV surveillance in the United States (Eidson et al., 2001; Kramer et al. 2001).

Various approaches to detecting WNV, including virus isolation, viral RNA detection, and immunohistochemistry (IHC), have proved effective (Steele et al., 2000). In order to maximize the likelihood of detection, however, information on tissue-specific viral titers associated with WNV infection is needed. To date, this work has been restricted to naturally and experimentally infected American crows and experimentally infected blue jays. One study of naturally infected American crows used TaqMan reverse transcription–polymerase chain reaction (RT-PCR) and plaque assay to compare WNV titers in six different tissues and found brain tis-

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sue to be the most sensitive target organ (Panella et al., 2001). A second study using TaqMan RT-PCR on tissues of naturally infected American crows found kidney, and secondarily brain, to be infected most consistently (Kramer and Bernard, 2001). Virus isolation and RT-PCR, performed on kidney and heart tissue from naturally infected birds in the New York area, were consistently positive (Steele et al., 2000). In this same study, brain was the least sensitive tissue for IHC in American crows, fish crows, and black-billed magpies (Steele et al., 2000). In experimental infection of blue jays, the highest viral titers were observed in lung at 9.2 $\log_{10}$ plaque forming units/0.5 cm^3 (Komar et al., 2003). Immunohistochemistry, virus isolation, and RT-PCR performed on replicate tissue samples from a variety of naturally infected avian species revealed that heart, kidney, and spleen were consistently positive by all three diagnostic tests (Steele et al., 2000).

Studies on the pathology of WNV in avian species have described a range of gross and microscopic lesions present in infected birds. Gross hemorrhage of the brain, splenomegaly, meningoencephalitis, and myocarditis were the most prominent lesions noted in birds found dead at the beginning of the WNV epidemic (Steele et al., 2000). Subsequent studies noted similar lesions in experimentally infected blue jays, crows, chickens, and turkeys, as well as naturally infected owls (Senne et al., 2000; Swayne et al., 2000; Fitzgerald et al., 2003; Weingartl et al., 2004; Wünschmann et al., 2004). No pathognomonic lesions for WNV have been described, however, and lesions between species are not consistent.

West Nile virus was first detected in Georgia, USA, in the summer of 2001. During that year, blue jays represented 30% of all dead bird submissions from Georgia, while American crows represented 17%; 50% of all the avian WNV isolates were from blue jays, while 43% were from American crows (SCWDS, unpubl. data). These results indicate that blue jays are an effective indicator species for WNV and may be regionally important in surveillance efforts. This may be especially true in locations where American crows are not abundant or adequately represented in dead bird submissions.

The objectives of the study were to describe the gross and microscopic pathology associated with natural WNV infection of blue jays and to determine the most appropriate blue jay tissues to be used for virus isolation, reverse transcription–nested polymerase chain reaction (RT-nPCR), and IHC techniques. The current study builds on the knowledge of the pathogenesis of WNV infection in birds by describing in detail the lesions associated with WNV infection, identifying sites of viral replication, and determining appropriate diagnostic tests for blue jays naturally infected with WNV.

MATERIALS AND METHODS

Bird selection and sample collection

Fifty-nine blue jays, of 475 submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS) during May to September 2001 as part of Georgia's WNV surveillance program, were selected for evaluation. Those birds with the least postmortem autolysis were chosen. Necropsies were performed in a class II safety cabinet, and birds were handled and samples collected by procedures approved by the Animal Care and Use Committee at the University of Georgia (A2000-10071-M2). Brain stem/cerebellum and heart were aseptically collected for initial screening by virus isolation. In addition, duplicate 0.5 cm^3 samples of heart, lung, liver, kidney, brain, spleen, flight muscle, and bursa were taken aseptically from each bird. One set of these samples, for use in virus titrations and RNA detection by RT-nPCR, was placed in separate microcentrifuge tubes containing 0.5 ml of BA-1 medium (minimal essential medium, 0.05 M Tris pH 7.6, 1% bovine serum albumin, 0.35 g/l sodium bicarbonate, 100 units/ml penicillin, 100 mg/ml streptomycin, 1 mg/ml Fungizone) and stored at −70 C until analyzed (Langevin et al., 2001). The second set of samples was placed in 10% buffered formalin for 24 hr and embedded in paraffin for histopathology and IHC.

Initial virus isolation and RT-nPCR results indicated that 36 of the 59 blue jays chosen

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for evaluation were WNV positive. Twenty WNV-positive and five WNV-negative birds were then randomly chosen to compare virus isolation and quantification, RT-nPCR, and IHC techniques. Tissues from six of the positive birds and all five negative birds used for comparison of techniques were also evaluated for microscopic pathology.

Gross and microscopic pathology

Gross examination was performed on brain, heart, lung, liver, kidney, spleen, bursa, gastrointestinal tract, skeletal muscle, pancreas, reproductive tract, and skin. Gross examination findings were categorized into six groups: calvarial hemorrhage, presence of myocardial lesions, presence of splenomegaly, pulmonary congestion, body condition, and presence of trauma. Myocardial lesions included pallor and hemorrhage. Body condition was categorized as emaciated, thin, or good. A chi-square test was used to determine whether differences in the prevalence of gross findings in the 36 positive and 23 negative birds were significant.

Sections of formalin-fixed paraffin embedded tissues (3–5 μm thick) were stained with hematoxylin and eosin. Five negative and six positive birds with minimal autolysis were chosen for microscopic evaluation, since autolysis can obscure subtle lesions. Tissues evaluated histologically and immunohistochemically from these birds included brain, heart, lung, liver, kidney, and spleen.

Initial screening using virus isolation and RT-PCR

Virus isolation was performed on brain stem/cerebellum and heart tissues to screen the 59 sample birds for WNV infection. All virus work was performed under biosafety level 3 conditions. Tissues were macerated with a plastic tissue grinder in BA-1 and centrifuged at 7,200 × G for 5 min. Supernatant of homogenized samples (100 μl) was placed on 3-day-old Vero cell (African green monkey kidney) monolayers in 12-well cell culture plates (Corning, Acton, Massachusetts, USA). Each well contained 2 ml of maintenance medium consisting of 2% antibiotic solution (100 units/ml penicillin, 100 mg/l streptomycin, and 100 mg/ml Fungizone), 3% fetal bovine serum, and 95% minimum essential medium with nonessential amino acids. Monolayers were incubated at 37 C and observed daily for cytopathic effect (CPE) for 7 days. If CPE was not observed at 7 days, the sample was passaged to new cells and observed for an additional 7 days.

Reverse transcription–polymerase chain reaction was performed to confirm WNV in samples with CPE. Total RNA was extracted from the supernatant using a QIAamp viral RNA Mini Kit (Qiagen Inc., Valencia, California, USA) following the manufacturer's protocol. Reverse transcription of RNA to cDNA and subsequent primary amplification were carried out in a single-tube reaction. Briefly, 2 μl of each RNA sample was added to individual 0.5-ml thin-walled PCR tubes (USA Scientific Inc., Ocala, Florida, USA) containing 48 μl of premix, consisting of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 1.6 mM beta-mercaptoethanol, 0.25 mM of each of the deoxyribonucleoside triphosphates, 200 ng primers (WNV 897-F 5′-GCYGTCATTGGWTGGATG and WNV 1195-R 5′-TCRTTGTGRGCTTCWCCCAT), 3 U avian myeloblastoma virus reverse transcriptase and 1.5 U *Taq* polymerase (Promega Corp., Madison, Wisconsin, USA). Negative controls to detect possible cross-contamination consisted of 2.0 μl RNase-free water as template and 48 μl premix. Positive reaction controls consisted of RNA extracted from the WNV isolate from Egypt (Eg101).

Reaction mixtures were incubated at 43 C for 15 min then subjected to 40 cycles of 94 C for 45 sec, 50 C for 60 sec, and 72 C for 75 sec using a PTC-100™ Thermal Cycler (MJ Research, Inc., Waltham, Massachusetts, USA). Reaction products were separated by electrophoresis through a 1.5% agarose gel, stained in ethidium bromide, and visualized under ultraviolet light. Visualization of a 298-bp product indicated that WNV RNA was present in the original sample.

Virus titrations

End point titrations were performed on tissues from the 20 WNV-positive and five negative birds identified by virus isolation during the initial screening process. Frozen tissues were thawed and macerated in BA-1 with a glass tissue grinder and centrifuged at 7,200 × G for 5 min. Virus titrations were performed in 96-well plates containing Vero cells with eight replicated wells per dilution (Lennette et al., 1995). Titrations were begun at 10^{-2}, since toxicity was encountered at lesser dilutions. Plates were incubated at 37 C for 7 days, at which time the 50% tissue culture infective dose ($TCID_{50}$) was determined using the Spearman-Kärber method (Finney, 1964).

RNA extraction and reverse transcription nested polymerase chain reaction (RT-nPCR)

Nested RT-PCR was performed on all tissues of the 20 positive and five negative blue jays. Total RNA was extracted from 125 μl of ground tissue in an RNase-free environment using an

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RNeasy Mini Kit (Qiagen Inc., Valencia, California, USA) following the manufacturer's protocol. The reverse transcription and primary reaction methods were the same as those used for initial screening; however, WNV-310F 5′-TSAACAAACAAACAGCRATGAA and WNV-1637R 5′-AGGTTSAGRTCCATRAACCA forward and reverse primers were used for the primary reaction, and primers WNV 897-F 5′-GCYGTCATTGGWTGGATG and WNV 1195-R 5′-TCRTTGTGRGCTTCWCCCAT were used for the nested reaction. Methods and materials for the nested reaction were the same as for the initial screening RT-PCR with the following exceptions: 1.0 μl of the first-stage amplification products was added to individual 0.5-ml thin-walled PCR tubes containing 49 μl of the premix, and the reaction mixtures were subjected to 35 cycles of 94 C for 45 sec, 52 C for 45 sec, and 72 C for 60 sec using a PTC-100™ Thermal Cycler (MJ Research).

The RT-nested PCR (RT-nPCR) protocol used in this project was designed to accurately detect WNV RNA in avian tissues. Published sequences from representative WNV isolates (GenBank accession numbers AF260968, AF260967, AF202541, and AF196835) were aligned using Sequencher version 4.1 (Gene Codes Corp., Ann Arbor, Michigan, USA). The degenerate primers were designed to amplify a fragment spanning the premembrane gene from conserved regions of these alignments.

The sensitivity of the RT-nPCR was determined by comparing the endpoint titration of WNV stock (Georgia isolate DES-01-107) in Vero cell culture with the endpoint dilution at which viral RNA was detected by RT-nPCR. Tenfold dilutions of virus were prepared. The endpoint titration in cell culture was $10^{6.45}$ $TCID_{50}$/25 μl. The viral titers as determined by RT-nPCR were $10^{5.2}$ $TCID_{50}$/25 μl after first-stage amplification, and $10^{8.0}$/25 μl after nested amplification. Specificity was examined by testing viral RNA extracted from related flaviviruses (St. Louis encephalitis virus and Dengue viruses 1–4) and from unrelated North American arboviruses that are found in avian species (Eastern equine encephalitis virus, Western equine encephalitis virus, and Highlands J virus). The RT-nPCR procedure performed on these samples did not result in observable amplification.

Immunohistochemistry

Immunohistochemistry was performed on paraffin embedded tissues as previously described (Gottdenker et al., 2003). The primary antibody was a rabbit α-WNV polyclonal, which was diluted 1:500 (Bioreliance, Rockville, Maryland, USA) and applied for 1 hr at room temperature.

TABLE 1. Prevalence of various gross findings in birds that were West Nile virus (WNV) positive (n = 36) or negative (n = 23) as determined by virus isolation.

	Number of positive birds with lesion (%)	Number of negative birds with lesion (%)
Calvarial hemorrhage	13 (36.1)	10 (43.5)
Myocardial lesions	5 (13.9)	2 (8.7)
Splenomegaly[a]	17 (47.2)	2 (8.7)
Pulmonary congestion	2 (5.5)	11 (47.8)
Poor body condition[a]	22 (61.1)	3 (13.04)
Trauma	3 (8.3)	4 (17.4)

[a] Splenomegaly and poor body condition were statistically significant lesions in WNV-positive birds ($p \leq 0.005$) using a chi-square analysis.

RESULTS

Gross pathology

Splenomegaly and poor body condition were the most consistent gross findings among positive birds (Table 1).

Microscopic pathology

Histopathologic findings are summarized in Table 2. The most consistent histopathologic findings in the tissues of WNV-positive blue jays were mononuclear leukocytosis and epicarditis/myocarditis. Although leukocytosis was most easily observed within the lung, similar large mononuclear leukocytes were noted within vessels in all organs.

Cardiac lesions were mild. The most common changes were the presence of lymphoplasmacytic infiltrates with fewer macrophages within the myocardial interstitium and the presence of macrophages with fewer lymphocytes and plasma cells in the epicardium. Epicardial lesions were most severe around the base of the heart and at the junction of the atria and ventricles. Endocardial inflammation, when present, was very mild.

Vascular and perivascular lesions were observed in the great vessels of the heart and in a few smaller pulmonary vessels.

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TABLE 2. Microscopic pathology results of blue jay tissues in which no autolysis was observed. Tissues were taken from blue jays that were WNV positive ($n = 6$) or negative ($n = 5$) as determined by virus isolation.

	Virus isolation positive birds with lesion	Virus isolation negative birds with lesion
Heart		
Endocarditis	3/6[a]	0/5
Myocarditis	5/6	0/5
Epicarditis	6/6	0/5
Myofiber degeneration	1/6	0/5
Ganglioneuritis	1/6	0/5
Liver		
Hepatitis		
Perivascular	1/6	0/5
Periportal	0/6	2/5
Random	0/6	1/5
Mixed	3/6	0/5
Piecemeal necrosis	2/6	0/5
Hemosiderosis		
Mild	1/6	1/5
Moderate	0/6	2/5
Severe	2/6	0/5
Congestion	2/6	0/5
Hemorrhage	1/6	0/5
Atrophy	0/6	1/5
Kidney		
Tubular degeneration	4/5	1/5
Urates	1/5	0/5
Ureteritis	0/5	1/5
Lung		
Peribronchial lymphocytosis	1/6	1/5
Congestion	0/6	1/5
Necrosis (mild)	1/6	0/5
Spleen		
Swollen RE cells[b]	4/4	0/3
Hypercellularity	4/4	0/3
Plasmacytosis	0/4	1/3
Lymphocyte apoptosis	2/4	0/3
Hemosiderosis	1/4	0/3
Brain		
Histopathologic lesions	0/6	0/5
Blood/blood vessels		
Leukocytosis (mononuclear)	5/6	0/5
Vasculitis	2/6	0/5
Perivasculitis	2/6	0/5

[a] Number of tissues with lesion/number examined.
[b] Reticuloenothelial.

Inflammation was present in the tunica media, tunica adventitia, and surrounding adipose tissue. The pulmonary vasculitis was associated with a focal area of air sacculitis and was probably not directly due to WNV infection.

In the spleen, reticuloendothelial cells were hyperplastic and diffusely swollen. Lymphoid tissue was also hyperplastic with numerous large plasma cells containing Russell bodies. Other findings included marked, multifocal lymphoid apoptosis and large deposits of hemosiderin within reticuloendothelial cells.

Kidneys from four of five WNV-positive birds had degeneration of single or multiple tubular epithelial cells within scattered proximal tubules. Inflammation was not a prominent feature in any of the kidneys examined.

Lung lesions were inconsistent. Changes included increased numbers of peribronchial and perivascular lymphocytes and plasma cells and scattered, small, necrotic foci.

The most common lesion observed in the livers of WNV-positive birds was a lymphoplasmacytic infiltrate within perivascular and periportal areas. However, two WNV-negative birds had similar inflammatory infiltrates. Similarly, hemosiderin deposition within hepatocytes and Kupffer cells was noted in both WNV-positive and -negative birds.

No abnormalities were noted in any of the brains from the six WNV-positive birds.

Virus titration

Maximum virus titers are shown in Figure 1. The minimum detectable titer for all samples was $10^{3.1}$ $TCID_{50}$/ml. Mean ranked viral titers are shown in Figure 1. Brain, heart, and lung had the highest viral titers; liver, kidney, and muscle had the lowest. Viral titers from spleen and bursa fell between these two groups. Virus was not detectable in tissues from the five negative control blue jays.

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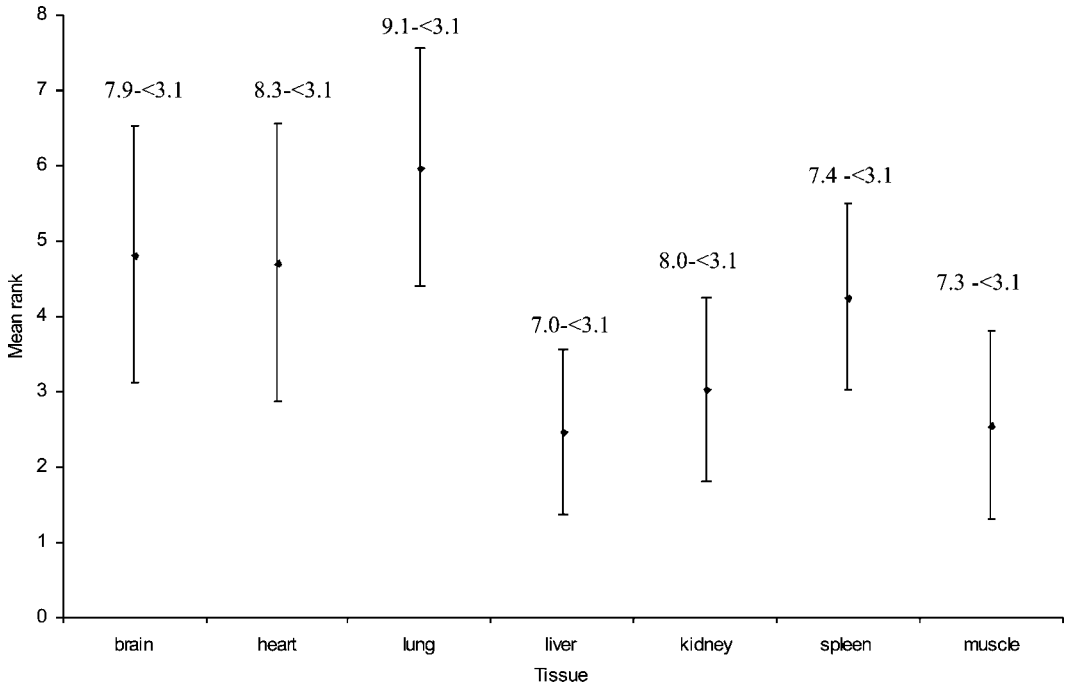


FIGURE 1. Comparison of mean rank and standard deviation for West Nile virus titers in seven different tissues of blue jays. Tissues were ranked according to West Nile viral titer within each bird, with the highest viral titer obtaining the highest rank. Values above standard deviation bars represent the range of viral titers for each tissue in $\log_{10}$ $TCID_{50}$/ml.

Reverse transcription–nested polymerase chain reaction

All tissues from WNV-positive blue jays were positive by RT-nPCR. Nested RT-PCR results were negative for all tissues in the five negative control blue jays.

TABLE 3. Immunohistochemistry (IHC) and reverse transcription nested polymerase chain reaction (RT-nPCR) results for eight tissues from 20 blue jays naturally infected with WNV as detected by virus isolation (brain and heart).

	IHC IHC pos/screen pos	RT-nPCR PCR pos/screen pos
Brain	10/20	20/20
Heart	18/20	20/20
Lung	15/20	20/20
Liver	16/20	20/20
Kidney	18/20	20/20
Spleen	12/20	20/20
Muscle	Not done	20/20
Bursa[a]	Not done	5/5

[a] For bursa, $n = 5$.

Immunohistochemistry

Results of IHC by individual tissues are given in Table 3. All 20 WNV-positive birds were also positive by IHC in at least one tissue. West Nile virus antigen was most often detected in the heart, kidney, liver, and lung. In the heart, staining ranged from multifocal to diffuse with antigen present (in decreasing order) in interstitial cells, myocardial fibers, and infiltrating macrophages. Staining was especially common within macrophages infiltrating epicardial adipose tissue. Antigen was also detected in the great vessels of the heart in a few birds within infiltrating macrophages and rarely in endothelial cells. In the kidney, antigen was present most often within resident interstitial cells and infiltrating macrophages and rarely within tubular epithelium; staining in the kidney was most often multifocal but sparse. In the liver, antigen was observed

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in virtually all Kupffer cells. Hepatocytes were never observed to contain antigen. A similarly generalized staining of splenic reticuloendothelial cells was observed. Staining was sparse but multifocal in the lung, with antigen observed in tissue macrophages and circulating monocytes. Staining was highly variable in brain. Seven birds had staining of capillary associated cells in the brain, which ranged from mild and scattered (three birds) to diffuse (four birds). The identity of these cells is not known, but possibilities include astrocytes, microglia, or extravasating macrophages. Antigen was also observed in neurons, particularly in and around the optic lobe, and glial cells. In four birds, the only WNV-positive cells in the brain were intravascular and/or perivascular monocytes and macrophages in the choroid plexus.

DISCUSSION

Detectability of WNV varies by tissue and is dependent on the diagnostic test employed. Lung, brain, and heart tissues of naturally infected blue jays in this study contained the highest WNV titers of the eight tissues examined. For blue jays, dead bird surveillance efforts using virus isolation can be limited to one or more of these tissues. The authors have found brain tissue to be the most practical for use with virus isolation, since it is simple to remove from the carcass and is easily homogenized.

Gross and microscopic pathology were the least effective procedures for WNV diagnosis in blue jays. Although all of the gross lesions observed by Steele et al. (2000) were seen in one or more of the blue jays in this study, only splenomegaly and body condition were significantly correlated with WNV infection. These nonspecific lesions are not diagnostic for WNV infection, however, since other infectious agents may cause similar gross lesions. In short, WNV cannot be accurately diagnosed based on gross pathology in blue jays.

West Nile virus antigens were most often detected in kidney, heart, liver, and lung tissue by IHC, making them the most optimal tissues for use with this procedure. As many labs do not have BSL-3 facilities, IHC is an appropriate alternative to virus isolation. IHC may also be employed for relatively fast turnaround of results since a diagnosis may be reached within 48 hr of tissue collection. The success of IHC was shown in this study to be highly dependent on the tissues tested. Weingartl et al. (2004) noted that viral antigen in experimentally infected crow and blue jay brains was not easily detected by IHC; this is consistent with our findings. Care must be taken in interpreting IHC of the heart tissue since staining of myofibers often has a paintbrush appearance that could easily be misinterpreted as background. Renal tubular epithelium often stains nonspecifically, but this is not difficult to differentiate from true staining. Also, owing to variability in staining between tissues, the authors recommend using at least three of the optimal tissues when performing surveillance to increase sensitivity.

Nested RT-PCR proved to be the most sensitive diagnostic test applied in this study and did not detect viral RNA in any of the tissues from five WNV-negative blue jays. The nested protocol allows results to be determined within 24 hr of tissue collection. The equipment required for RT-nPCR is expensive; however, the speed and sensitivity of the test exceeded the capabilities of both virus isolation and IHC. This advantage is even more apparent with currently used real-time PCR formats that can provide both rapid diagnosis and quantitative results (Lanciotti et al., 2000). As with virus isolation, brain tissue may be the most practical tissue for PCR because of ease of collection and maceration.

In other studies of WNV infection in birds, a triad of lesions (histiocytic to lymphoplasmacytic epicarditis/myocarditis, lymphoplasmacytic encephalitis, and renal tubular degeneration) has been described as being highly suggestive of WNV infection (Garmendia et al., 2000; Steele et al.,

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2000; Swayne et al., 2001). However, histopathologic lesions of encephalitis were not observed, and renal lesions were uncommon in the blue jays in this study, similar to reported findings in blue jays, crows, chickens, and turkeys (Senne et al., 2000; Swayne et al., 2001; Weingartl et al., 2004).

One lesion that was commonly noted in the blue jays was the presence of numerous large mononuclear cells in blood. This finding has not been previously reported in any avian species with WNV infection. Mild hepatocellular necrosis, observed in two WNV-positive blue jays in this study, has been described by Steele et al. (2000) and Weingartl et al. (2004). Several lesions observed in the blue jays, including hepatitis and hemosiderosis in liver and spleen, were interpreted to be unrelated to WNV infection since they were seen in both WNV-positive and -negative birds. Urates were noted in the kidney of one WNV-positive bird, but this is merely indicative of dehydration.

Immunohistochemistry results highlight the fact that gross and histopathologic lesions are poor indicators of the presence of viral antigen. Viral antigen can be, and often is, present in large amounts in the absence of corresponding gross or histopathologic lesions. Even in cases where inflammation was present in a tissue such as heart, the majority of the staining occurred in histologically normal areas with only small amounts of antigen noted in inflamed areas.

The high WNV titers present in these blue jay tissues reinforces the need for personal protection when handling the birds and performing postmortem exams. The high titers also illustrate the potential for blue jay tissues to be a source of WNV infection if ingested by scavenger species.

Immunohistochemistry, virus isolation, and RT-nPCR are all useful techniques in WNV surveillance. We have shown, however, that when employing these techniques, tissue selection is critical for immunohistochemistry (heart, kidney, liver, and lung were best) and virus isolation (based on tissue titer, brain, heart, and lung were best). With RT-nPCR, however, it seems possible to detect viral RNA in a variety of tissues of positive birds, allowing the investigator to choose the tissue based on ease of collection.

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