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NOTES

Comparison of Immunohistochemistry and Virus Isolation for Diagnosis of West Nile Virus

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Immunohistochemistry and virus isolation were performed on 1,057 birds. Immunohistochemistry, virus isolation, or both found 325 birds to be West Nile virus positive. Of these, 271 were positive by both methods. These results indicate that virus isolation and immunohistochemistry are approximately equal in their ability to detect West Nile virus.

West Nile virus (WNV) is a member of the family *Flaviviridae*, genus *Flavivirus*. It is transmitted by mosquito vectors to a variety of avian hosts and incidentally to horses and humans (1). West Nile virus was first reported in the United States in 1999 in New York, where it was associated with an outbreak that killed hundreds of birds (2–4).

Since West Nile virus is a zoonotic agent and mortality in birds has usually preceded human infection and death, primary detection of virus in birds is an important part of surveillance for this virus (5, 6). Previous studies have used immunohistochemistry (IHC) and virus isolation (VI) to diagnose West Nile virus (8, 9), but there has not been a large-scale comparison of these two methods. This study compares the results of virus isolation and immunohistochemistry for 1,057 birds.

Birds were voluntarily submitted to the Southeastern Cooperative Wildlife Disease Study through state and local health departments in Georgia. Necropsies were performed on all birds in a biosafety cabinet. Liver, kidney, brain, and heart tissues were placed in 10% buffered formalin, and aseptically obtained brain and heart tissues were collected in microcentrifuge tubes containing BA-1 solution. Immunohistochemistry and virus isolation were performed as previously described (7). A "positive result" using IHC was defined as a bird that had intracellular staining in one or more tissues. "Equivocal results" for immunohistochemistry were defined as those which were impossible to judge as positive or negative.

Submitted birds represented at least 78 species (Table 1), 16 of which were positive for West Nile virus. Comparison of immunohistochemistry and virus isolation results yielded a 95% agreement rate (990/1,039). The 18 birds with an equivocal result by IHC were excluded from this total.

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For immunohistochemistry, brain, heart, kidney, and liver tissues were available for most birds (97%, 97%, 87%, and 88%, respectively). In birds that were IHC positive, brain tissue was positive in 118/285 cases (41%), heart tissue was positive in 279/285 cases (98%), kidney tissue was positive in 250/267 cases (94%), and liver tissue was positive in 240/266 cases (90%).

Staining patterns on immunohistochemistry were consistent within each tissue (Fig. 1). In liver tissue, staining was confined to Kupffer cells. In kidney tissue, staining was multifocal and centered around collecting ducts. Cells that stained appeared to be a combination of macrophages, tubular epithelial cells, and cells of unknown origin. In heart tissue, staining ranged from faint and focal to overwhelming and diffuse and was most commonly seen in myofibers and infiltrating macrophages. Staining in brain tissue was usually focal and often rare. These foci consisted of a positive neuron(s) surrounded by positive glial cells. Focal or multifocal staining of Purkinje cells and mild gliosis in the cerebellum were sometimes observed.

In 311 cases that were positive by VI, most cases (68%) were positive in both brain and heart tissue. However, 23% were positive only in brain tissue and 6% were positive only in heart tissue. In one case, only a cloacal swab was positive, and in four cases, results were recorded as positive without regard to tissue.

The high agreement rate (95%) between virus isolation and immunohistochemistry indicates that the two methods are approximately equal regarding ability to detect West Nile virus. Some cases provided equivocal results by immunohistochemistry such as those with heavy background, severe autolysis, very weak staining, or staining in unusual patterns or tissues.

Virus isolation appears slightly more sensitive in that it detected 40 cases that were negative or equivocal on immunohistochemistry whereas immunohistochemistry detected only 14 cases that were negative on virus isolation. Virus isolation has the additional advantage of allowing follow-up with reverse

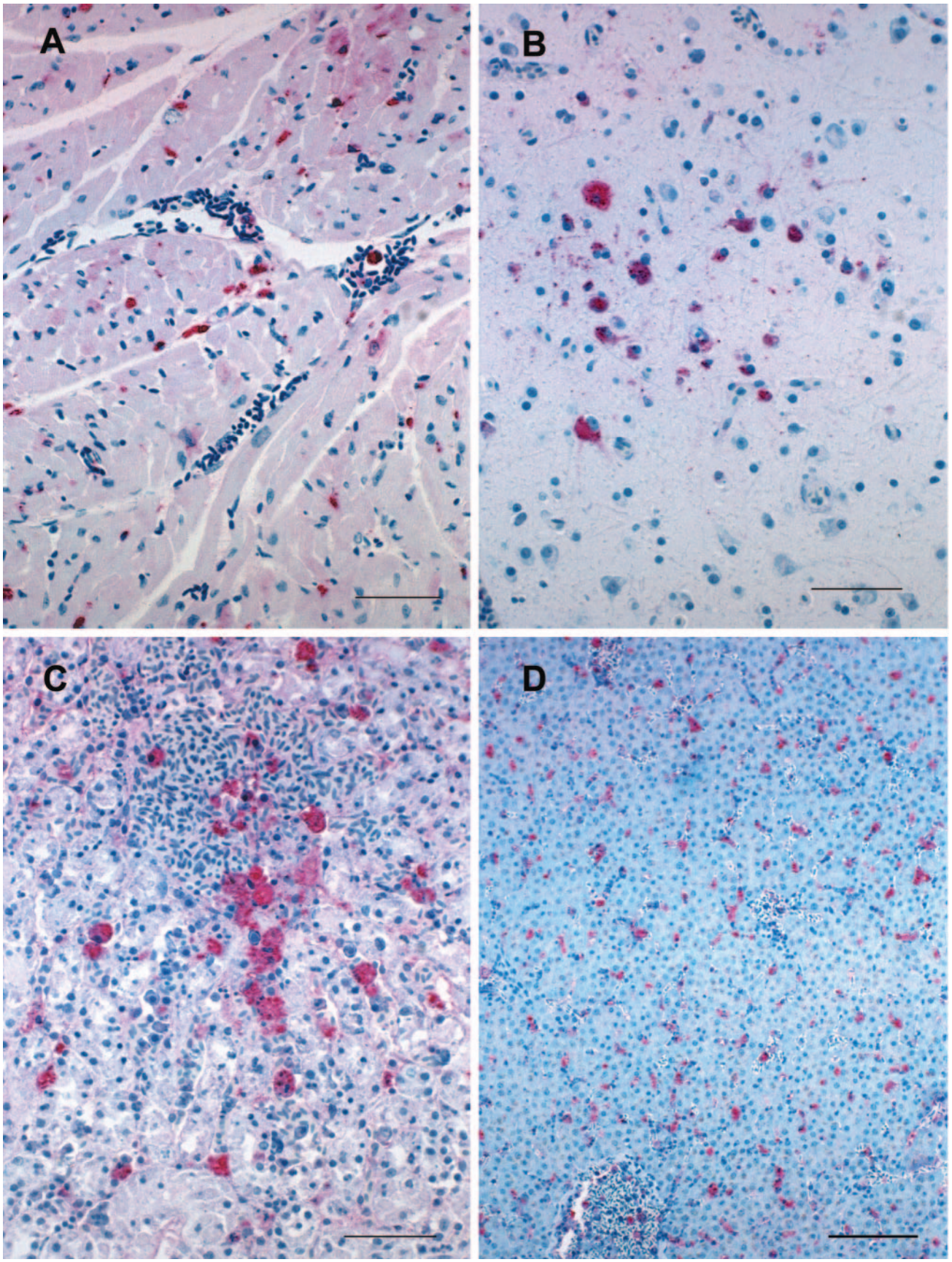

FIG. 1. Typical immunohistochemical staining patterns using Fast Red chromogen and hematoxylin counterstain (clockwise from top left). A. Section of heart demonstrating positive interstitial and mononuclear cells and myofibers. Bar, 35 μm. B. Section of cerebrum with positive neurons surrounded by positive glial cells. Bar, 35 μm. C. Section of kidney showing positive mononuclear cells in the interstitium, peritubular capillaries, and a large blood vessel in a collecting duct area. Bar, 35 μm. D. Section of liver with positive Kupffer cells. Bar, 75 μm.

TABLE 1. Species of birds tested for WNV

Order	No. of birds				% Agreement[b]	Species[a]
	Submitted	Positive by:		Equivocal by IHC		
		VI	IHC			
Anseriformes	1	0	0	0	100	Mallard, *Anas platyrhynchos*
Apodiformes	1	0	0	0	100	Chimney swift, *Chaetura pelagica*
	2	0	0	0	100	Hummingbird, unspecified
	3	0	0	0	100	Ruby-throated hummingbird, *Archilochus colubris*
Caprimulgiformes	3	0	0	0	100	Common nighthawk, *Chordeiles minor*
	1	0	0	0	100	Nightjar, unspecified
Charadriiformes	1	0	0	0	100	American woodcock, *Scolopax minor*
	1	0	0	0	100	Spotted sandpiper, *Actitis macularia*
Ciconiformes	1	0	0	0	100	American bittern, *Botaurus lentiginosus*
	1	0	0	0	100	Black vulture, *Coragyps atratus*
	1	0	0	0	100	Great egret, *Ardea alba*
	1	0	0	0	100	Night heron, unspecified
	1	0	0	0	100	Turkey vulture, *Cathartes aura*
Columbiformes	1	0	0	0	100	Dove, unspecified
	2	0	0	0	100	Eurasian collared dove, *Streptopelia decaocto*
	24	0	0	0	100	Mourning dove, *Zenaida macroura*
	20	0	4	4	75	**Rock dove**, ***Columba livia***
Cuculiformes	4	0	0	0	100	Yellow-billed cuckoo, *Coccyzus americanus*
Falconiformes	1	0	0	0	100	American kestrel, *Falco sparverius*
	2	0	0	0	100	Broad-winged hawk, *Buteo platypterus*
	27	1	1	0	100	**Cooper's hawk**, ***Accipiter cooperii***
	8	2	0	0	75	**Osprey**, ***Pandion haliaetus***
	4	0	0	0	100	Red-shouldered hawk, *Buteo lineatus*
	12	1	1	0	100	**Red-tailed hawk**, ***Buteo jamaicensis***
	12	0	0	0	100	Sharp-shinned hawk, *Accipiter striatus*
Galliformes	8	0	0	0	100	Domestic chicken, *Gallus gallus*
Gruiformes	2	0	0	0	100	American coot, *Fulica americana*
	2	0	0	0	100	King rail, *Rallus elegans*
	1	0	0	0	100	Sora, *Porzana carolina*
	1	0	0	0	100	Virginia rail, *Rallus limicola*
Passeriformes	240	130	123	2	93	**American crow**, ***Corvus brachyrhynchos***
	2	0	0	0	100	American goldfinch, *Carduelis tristis*
	10	0	0	0	100	American robin, *Turdus migratorius*
	1	0	0	0	100	Bachman's sparrow, *Aimophila aestivalis*
	2	0	0	0	100	Black-and-white warbler, *Mniotilta varia*
	1	0	0	0	100	Blackbird, unspecified
	420	165	147	9	93	**Blue jay**, ***Cyanocitta cristata***
	13	0	0	0	100	Boat-tailed grackle, *Quiscalus major*
	5	0	0	0	100	Brown-headed cowbird, *Molothrus ater*
	17	0	1	0	94	**Brown thrasher**, ***Toxostoma rufum***
	2	0	0	0	100	Carolina wren, *Thryothorus ludovicianus*
	1	0	0	0	100	Chipping sparrow, *Spizella passerina*
	26	2	2	1	96	**Common grackle**, ***Quiscalus quiscula***
	17	0	1	1	88	**Common yellowthroat**, ***Geothlypis trichas***
	9	0	0	0	100	Eastern bluebird, *Sialia sialis*
	1	0	0	0	100	Eastern kingbird, *Tyrannus tyrannus*
	6	0	0	0	100	European starling, *Sturnus vulgaris*
	1	0	0	0	100	Field sparrow, *Spizella pusilla*
	1	0	0	0	100	Flycatcher, unspecified
	1	0	0	0	100	Golden-crowned sparrow, *Zonotrichia atricapilla*
	2	0	0	0	100	Grackle, unspecified
	25	0	0	0	100	Gray catbird, *Dumetella carolinensis*
	1	0	0	0	100	Gray-cheeked thrush, *Catharus minimus*
	3	1	0	0	67	**Hermit thrush**, ***Catharus guttatus***
	1	0	0	0	100	Hooded warbler, *Wilsonia citrina*
	3	0	0	0	100	House sparrow, *Passer domesticus*
	1	0	0	0	100	House wren, *Troglodytes aedon*
	1	0	0	0	100	Indigo bunting, *Passerina cyanea*
	12	4	3	0	92	**Northern cardinal**, ***Cardinalis cardinalis***
	14	2	1	0	93	**Northern mockingbird**, ***Mimus polyglottus***
	1	0	0	0	100	Northern water-thrush, *Seiurus noveboracensis*
	1	0	0	0	100	Orchard oriole, *Icterus spurius*
	1	0	0	0	100	Ovenbird, *Seiurus aurocapillus*
	3	0	0	0	100	Red-eyed vireo, *Vireo olivaceus*

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TABLE 1—*Continued*

Order	No. of birds				% Agreement[b]	Species[a]
	Submitted	Positive by:		Equivocal by IHC		
		VI	IHC			
	5	0	0	0	100	Red-winged blackbird, *Agelaius phoeniceus*
	2	0	0	0	100	Scarlet tanager, *Piranga olivacea*
	3	0	0	0	100	Swainson's thrush, *Catharus ustulatus*
	1	0	0	0	100	Swallow, unspecified
	1	0	0	0	100	Swamp sparrow, *Melospiza georgiana*
	2	0	0	0	100	Thrush, unspecified
	2	0	0	0	100	Tufted titmouse, *Baeolophus bicolor*
	1	0	0	0	100	White-eyed vireo, *Vireo griseus*
	1	0	0	0	100	White-throated sparrow, *Zonotrichia albicollis*
	1	0	0	0	100	Winter wren, *Troglodytes troglodytes*
	2	1	0	0	50	**Wood thrush**, ***Hylocichla mustelina***
	3	0	0	0	100	Yellow-rumped warbler, *Dendroica coronata*
Piciformes	1	0	0	0	100	Northern flicker, *Colaptes auratus*
	1	0	0	0	100	Red-bellied woodpecker, *Melanerpes carolinus*
	1	0	1	0	0	**Red-headed woodpecker**, ***Melanerpes erythrocephalus***
	3	0	0	0	100	Yellow-bellied sapsucker, *Sphyrapicus varius*
Psittaciformes	4	1	0	0	75	**Parakeet**, **unspecified**
Strigiformes	3	0	0	0	100	Barn owl, *Tyto alba*
	8	1	0	0	88	**Barred owl**, ***Strix varia***
	10	0	0	0	100	Eastern screech-owl, *Otus asio*
	8	0	0	1	100	Great horned owl, *Bubo virginianus*
Total	1,057	311	285	18		

[a] Species positive for WNV by either IHC or VI are indicated by boldface.
[b] Total no. of cases in agreement/total no. of cases = 95% (Cases with equivocal IHC results were excluded from the total no. of cases).

transcription-PCR. This confirms the presence of West Nile virus specifically and allows for identification of other viruses. Our current immunohistochemical technique uses a polyclonal antibody that cross-reacts with Saint Louis encephalitis virus. Therefore, positive diagnosis of West Nile virus requires follow-up with some other method of identification or use of a monoclonal antibody. Although none of the birds in this study were found to have Saint Louis encephalitis virus, Newcastle disease virus, Highlands J virus, and Eastern equine encephalitis virus were isolated from one, two, and three birds, respectively. The final major advantage of virus isolation is that it allows for quantitative analysis of virus in tissues.

Advantages of immunohistochemistry are a faster turnaround time (typically 2 days versus 7 to 14 days for VI) and opportunity for histopathologic examination of tissues. This allows for identification of confounding factors that might have contributed to, or even caused, death. The protocol is also easily adaptable to an automated immunostainer. Immunohistochemistry also requires less-specialized equipment, and biosafety level 3 facilities are not needed. There is less risk to laboratory personnel, since live virus is not present in formalin-fixed tissues. The main disadvantage is that results may be equivocal due to autolysis, nonspecific staining, or weak staining. Our results indicate that virus isolation still works on severely autolyzed tissue while immunohistochemistry results may be equivocal.

It is important to note that each test requires different tissues for optimal diagnostic ability. For virus isolation, brain tissue was positive in 92% of positive cases while heart tissue was positive in 75% of positive cases. By IHC, brain tissue was positive in only 40% of positive cases, whereas heart tissue was IHC positive in 96% of positive cases. Since it is possible to test multiple organs simultaneously using IHC, it is probably best to base any evaluation on several tissues rather than just one or two.

While combined use of immunohistochemistry and virus isolation may slightly improve diagnostic ability, it is not practical in terms of time or economics to use both methods for screening. The decision on which method to use may depend on availability of equipment and facilities, availability and training of personnel, and personal preference. With immunohistochemistry, laboratory personnel are not exposed to live virus beyond the initial sample collection and biosafety level 3 facilities are not required. However, we have used both methods successfully and safely and do not specifically favor one over the other.

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REFERENCES

1. **Burke, D. S., and T. P. Monath.** 2001. Flaviviruses, p. 1089–1092. *In* D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roizman, and S. E. Straus (ed.), Fields virology, 4th ed. Lippincott Williams & Wilkins, Philadelphia, Pa.

2. **Centers for Disease Control and Prevention.** 1999. Outbreak of West Nile-like viral encephalitis—New York, 1999. Morb. Mortal. Wkly. Rep. **48:**845–849.
3. **Centers for Disease Control and Prevention.** 1999. Update. West Nile-like viral encephalitis—New York, 1999. Morb. Mortal. Wkly. Rep. **48:**890–892.
4. **Centers for Disease Control and Prevention.** 1999. Update. West Nile-like viral encephalitis—New York, 1999. Morb. Mortal. Wkly. Rep. **48:**944–946.
5. **Eidson, M., N. Komar, F. Sorhage, R. Nelson, T. Talbot, F. Mostashari, R. McLean, and the West Nile Virus Avian Mortality Surveillance Group.** 1999. Crow deaths as a sentinel surveillance system for West Nile virus in the northeastern United States. Emerg. Infect. Dis. **7:**615–620.
6. **Eidson, M., L. Kramer, W. Stone, Y. Hagiwara, K. Schmit, and the New York State West Nile Virus Avian Surveillance Team.** 2001. Dead bird surveillance as an early warning system for West Nile virus. Emerg. Infect. Dis. **7:**631–635.
7. **Gottdenker, N. L., E. W. Howerth, and D. G. Mead.** 2003. Natural infection of a great egret (*Casmerodius albus*) with eastern equine encephalitis virus. J. Wildl. Dis. **39:**702–706.
8. **Panella, N. A., A. J. Kerst, R. S. Lanciotti, P. Bryant, B. Wolf, and N. Komar.** 2001. Comparative West Nile virus detection in organs of naturally infected American crows (*Corvus brachyrhynchos*). Emerg. Infect. Dis. **7:**754–755.
9. **Steele, K. E., M. J. Linn, R. J. Schoepp, N. Komar, T. W. Geisbert, R. M. Manduca, P. P. Calle, B. L. Raphael, T. L. Clippinger, T. Larsen, J. Smith, R. S. Lanciotti, N. A. Panella, and T. S. McNamara.** 2000. Pathology of a fatal West Nile virus infection in native and exotic birds during the 1999 outbreak in New York City. J. Vet. Pathol. **37:**208–224.