# Diagnostic Exercise

## From The Davis-Thompson Foundation*

Case #:**185**; Month: **March**; Year: **2022**

*Answer Sheet*

**Title: West Nile virus infection in a tufted duck (*Aythya fuligula*)**

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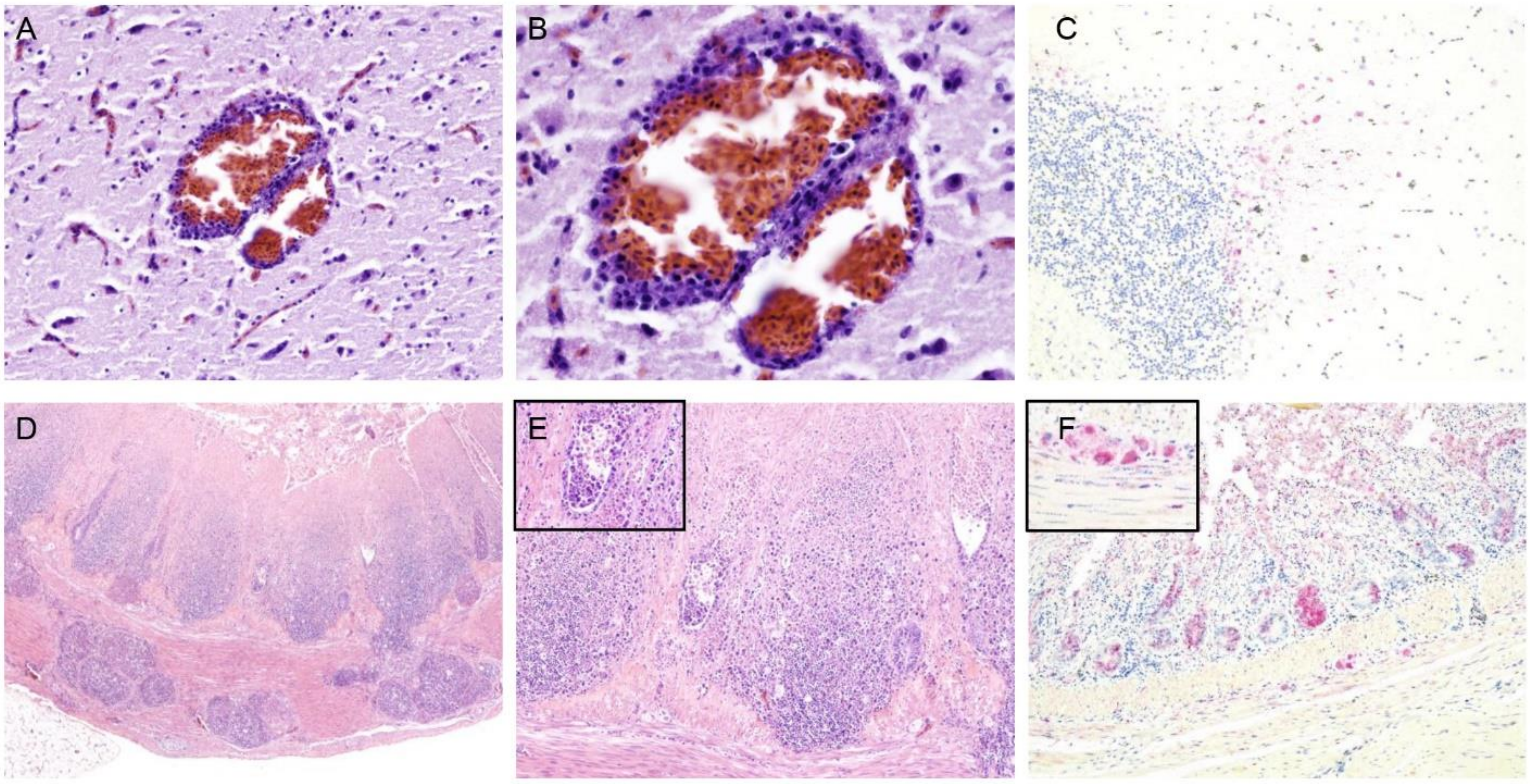

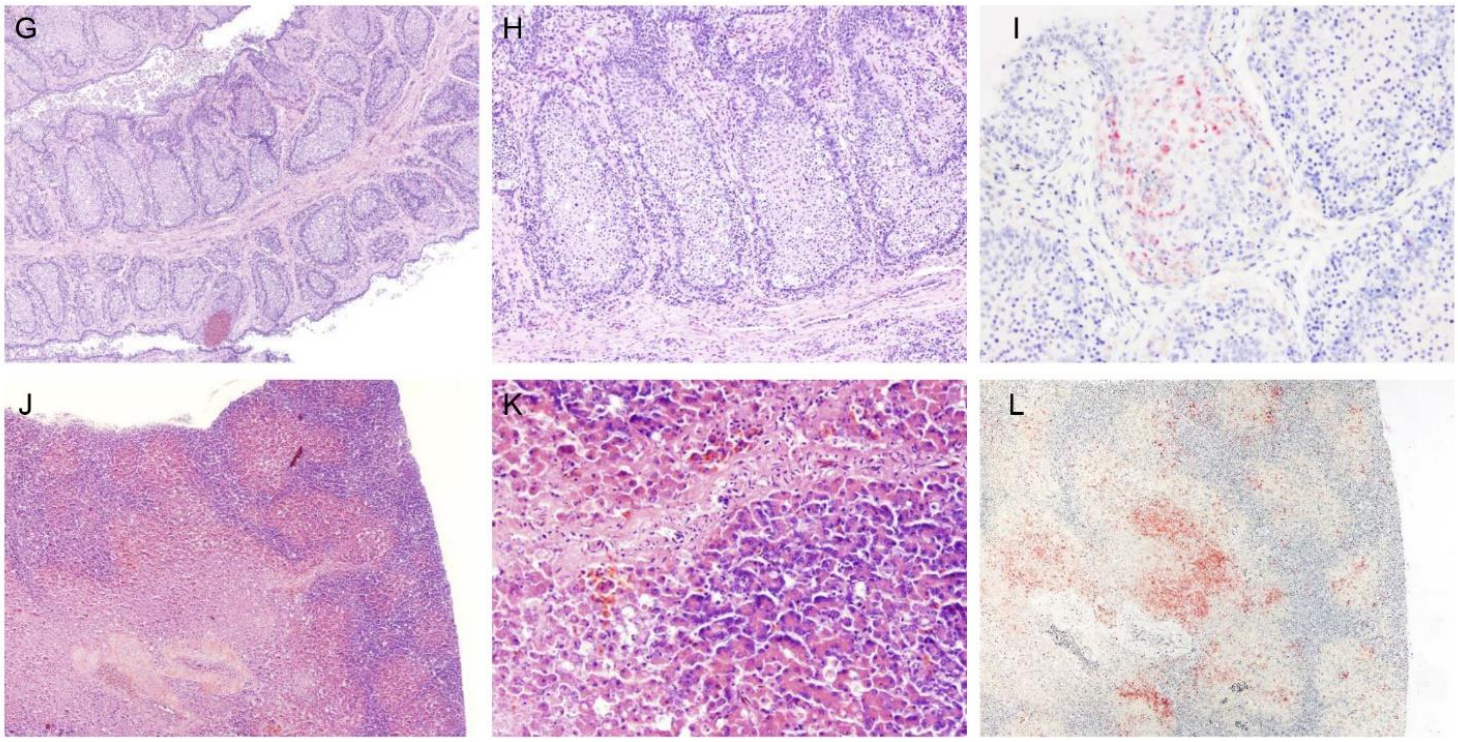


**Figure 2:** (A-C) Brain. Multifocal blood vessels within the neuroparenchyma have delicate perivascular cuffs composed of few lymphocytes. Increase in glial cells is noted in the neuroparenchyma (H&E, 200X and 400X). West Nile virus (WNV) antigen is detected in scattered neuronal bodies and processes (cerebellum, immunohistochemistry [IHC], Fast Red, 100X). (D-F) Small intestine. Multiple crypts are filled with abundant necrotic cellular debris and lined by attenuated epithelium. The Peyer's patches are hypocellular and contain karyorrhectic lymphocytes. H&E, 40X and 100X. WNV antigen is abundant in affected crypts, within depleted lymphoid follicles, smooth muscle cells and myenteric ganglia (F, inset). IHC, Fast Red, 100X. (G-I) Bursa of Fabricius. The lymphoid follicles are markedly hypocellular, containing karyorrhectic lymphocytes and tingible-body macrophages. H&E, 40X and 100X. WNV antigen is noted within depleted follicles and surface epithelium. IHC, Fast Red, 200X. (J-L) Pancreas. Multifocal pancreatic necrosis with abundant intralesional WNV antigen. H&E, 40X and 100X; IHC, Fast Red, 40X.

**Key Histologic Alterations:**
Brain: Perivascular cuffing of lymphocytes, gliosis, perivascular hemorrhage (not evident in the provided images).
Small Intestine: Crypt necrosis, epithelial attenuation/regeneration, lymphoid necrosis and depletion (Peyer's patches).
Bursa of Fabricius: Lymphoid necrosis and depletion.

**Morphologic Diagnoses:**
Brain: Encephalitis, lymphocytic, multifocal, mild, acute.
Small intestine: Enteritis, necrotizing, multifocal, marked, acute with severe lymphoid necrosis/depletion of Peyer's patches.
Bursa of Fabricius: Lymphoid necrosis and depletion, diffuse, marked, acute.
**Possible Causes:**
● West Nile virus

- Highly pathogenic avian influenza
- Exotic Newcastle Disease
- Eastern Equine Encephalomyelitis

**Lesions expected in other organs:**

Heart:

- Gross lesions: pale streaks or foci and hemorrhage in epicardium/myocardium
- Microscopic lesions: multifocal to coalescing myocardial necrosis, multifocal lymphoplasmacytic and histiocytic myocarditis

Pancreas:

- Gross lesions: multifocal white foci and/or hemorrhage
- Microscopic lesions: multifocal to coalescing foci of necrosis

Spleen:

- Gross lesions: splenomegaly, overall pallor
- Microscopic lesions: multifocal to coalescing necrotizing splenitis and lymphoid depletion

Kidneys:

- Gross lesions: pale foci or streaks, hemorrhage
- Microscopic lesions: variable non-suppurative, lymphoplasmacytic interstitial nephritis with tubular necrosis

Liver:

- Gross lesions: hepatomegaly, white foci
- Microscopic lesions: multifocal to coalescing hepatocellular necrosis, periportal lymphohistiocytic hepatitis

Eye:

- Gross lesions: Irregular region of white discoloration in the center of the fundus.
- Microscopic lesions: Lymphoplasmacytic pectenitis, choroiditis and iridocyclitis, and retinal necrosis and atrophy.

**Discussion:**

West Nile Virus (WNV) is a zoonotic arbovirus that belongs to the family *Flaviviridae (*genus *Flavivirus*), which consists of single-stranded positive-sense RNA viruses. Two lineages are considered the most prevalent: lineage-1 and lineage-2, although there are at least seven recorded.[1,9] WNV was first discovered in a febrile woman living in Uganda in 1937 and subsequently detected in a bird in 1953 near the Nile delta region. Between 1997-1999, a major outbreak of WNV in geese occurred in Israel and constituted the link for the subsequent spread to the US.[1,2] This *old world* virus later became a *new world* virus after being reported in New York in 1999.[3,6] Since then, over 300 species of domestic and wild birds have been affected worldwide, with new cases reported every year.[1]

WNV is maintained in an enzootic cycle involving mosquitos (primarily *Culex* and *Aedes* species) and birds. Mosquitos serve as the primary biological vector of WNV and are largely responsible for transmission between animal species. WNV has been isolated in other hematophagous parasites such as ticks, but their role in transmission is not well understood.[9] When a mosquito feeds on an infected bird (primary host), the virus then replicates in the salivary glands and midgut of the mosquito. Subsequently, infected mosquitos infect other birds/mammals during a bloodmeal. Horses and humans are dead-end hosts,[5,9] meaning that the viremia in these is not substantial enough to allow for effective transmission of the virus to an uninfected mosquito during a bloodmeal. In contrast, birds serve as reservoir hosts and remain viremic for four to seven days after the initial infection.[1] The virus can be detected in oral and cloacal swabs nine days post-infection and 14+ days in other tissues such as skin and intestines.[1] Susceptibility to infection and clinical signs vary depending on the species and individual factors.[9] High mortality is reported chiefly in crows and other Passeriformes.[6,9] Psittacines and Anseriformes are less affected, and Galliformes remain mostly asymptomatic.[1] Chickens are used as public health sentinels to monitor the transmission dynamics of WNV.[5] Species that make good sentinels are susceptible to infection but resistant to disease and produce adequate immune response that can be detectable by serological methods.[5]
The occurrence of WNV infection has a seasonal distribution (mainly dependent on the vector density),[6] with most cases reported during late summer and fall.[6,9] Nonetheless, WNV has also been detected during the winter, suggesting other modes of transmission are possible. Contact transmission has been demonstrated in birds of the Corvidae and Laridae families and may be relevant during large groupings of birds. Oral transmission depending on the bird species may also contribute to the spread of WNV. This is the case in birds of prey.[9] Infected prey animals, including mammals, reptiles, amphibians, or other birds, could serve as the source of infection for these birds after mosquito season is over. Migratory birds contribute to the transboundary spread of WNV across different regions of the world as they migrate from cooler to warmer climates.[6,7] In one study, the seroprevalence of WNV in birds across Bangladesh showed a higher percentage of tufted ducks affected (28.5%) compared to crows (12.5%).[5] This scenario has been also described for the Americas, with interhemispheric spread of WNV through migratory waves.

Clinical signs of WNV infection in avian species can include lethargy, ataxia, tremors, seizures, abnormal head posture, anisocoria, impaired vision, and sudden death.[1,2,9] The most affected organs are the brain, liver, kidney, and heart, but other tissues can be affected like lungs, pancreas, intestines, adrenal glands, and eyes. [1,2,7,9] The histologic lesions caused by WNV in birds such as hemorrhage, and multi-organ necrosis are like those caused by other viral diseases including highly pathogenic avian influenza, avian paramyxovirus 1 (APMV-1; Exotic Newcastle Disease)[3,4]or Eastern Equine Encephalitis virus (EEEV).[2]

The case presented here is a good example of the multi-organ/multi-systemic lesions expected with WNV, infection of which was confirmed via IHC and RT-qPCR. Other lesions found in this tufted duck included pancreatic necrosis and necrotizing ventriculitis. Interestingly, both intracellular and extracellular rod-shaped bacteria were also noted in the vasculature and interstitial histiocytes, mainly in the lungs and spleen. This finding is highly suggestive of sepsis, likely secondary to invasion

through the extensively necrotic intestinal lining. No bacterial culture or additional testing was performed to characterize the etiologic bacterial agent, however.

There are no licensed vaccines available to prevent outbreaks in avian species, although there are licensed vaccines available for horses that have been tested in birds.[1,9] The efficacy of vaccines explicitly developed for birds has not been comprehensively determined, as the response of some vaccines is inconsistent between different avian species, so mosquito control remains a critical method for reducing WNV in both animals and humans.

**References and Recommended Literature:**


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