Author's response to reviews

Title: Sensitivity and Specificity of Monoclonal and Polyclonal Immunohistochemical Staining for West Nile Virus in Various Organs from American Crows (Corvus brachyrhynchos)

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Author's response to reviews: see over
Dear Dr. Lolu da-Silva,

Thank you for considering this article, “Sensitivity and Specificity of Monoclonal and Polyclonal Immunohistochemical Staining for West Nile Virus in Various Organs from American Crows (Corvus brachyrhynchos)”, for publication in your journal. I have made the suggested reviewer revisions and would like to resubmit this revised version.

William Reisen asked what new information was learned from this study and how these techniques are useful for surveillance. No studies have been previously performed that directly compare the sensitivity and specificity of a monoclonal antibody to a polyclonal antibody for detection of WNV in naturally-infected American crows. Also, previous studies that have compared the use of an anti-WNV monoclonal antibody for immunohistochemical staining to various other tests, such as RT-PCR, have had conflicting results. In addition, these studies did not have as large a sample population as our study. I have attempted to better emphasize these points in the revised background section.

Most previous immunohistochemical studies have simply reported various organs as positive or negative but did not discuss the ease with which this diagnosis was made. In our study, we found that while sections of heart were consistently positive using the polyclonal antibody, it was often very difficult to appreciate the staining. By determining how easy it was to identify positive staining at low magnification, and the percentage of positive staining cells in each organ, our study identified the organs that were the most useful for the diagnosis of WNV. These factors had not previously been examined.

We agree that newer surveillance tests such as RT-PCR performed on cloacal and oral swabs are becoming more common; however, to our knowledge, many veterinary diagnostic laboratories are still using immunohistochemical staining methods for WNV surveillance in avian species. These laboratories may especially benefit from the results of this study.

I have addressed the point made by both reviewers that fresh tissues can be placed in a lysis buffer to inactivate the virus and thus, decrease the risk of human exposure. I have also addressed all but one of the minor essential revisions listed by Aaron Brault. The one revision I did not address was number 3 in which he wrote: “The initial isolation of the West Nile viral genotype introduced into North America was made from a migratory stork prior to the initiation of the commercial goose epizootic.” I did not make any revisions in the manuscript as my statement is only that the New York WNV strain is closely related to the virulent WN-Israel 1998 virus strain that was isolated from a goose. A later published reference by Malkinson, M. et al. entitled “Introduction of West Nile virus in the Middle East by Migrating White Storks” that was published in Emerging Infectious Diseases in April 2002, identified a WNV isolate in white storks that was closely related to both the New York WNV strain and the WN-Israel 1998 strain. These storks migrate through Europe and may have been the primary source of infection of geese in Israel. However, there is no reported direct migration of these storks to North America, and gulls, as well as other sea birds, have been suggested as primary routes of
transmission into the U.S. Due to this uncertainty of the initial source of the New York WNV strain, we did not want to expand on this topic.

Thank you again for your consideration. Please let me know if any additional clarifications are needed.

Sincerely,
Rebecca C. Smedley, DVM