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HEMATOLOGY RESULTS FROM EXPERIMENTAL EXPOSURE OF SANDHILL CRANES TO WEST NILE VIRUS

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West Nile virus (WNV), a Flavivirus, was introduced into New York City in 1999 (Centers for Disease Control 1999, Enserink 1999). In the past decade the virus has spread across the continental United States and southern Canada, resulting in large numbers of deaths among native bird species (Anderson et al. 1999, Calle et al. 2000). The U.S. Geological Survey (USGS) Patuxent Wildlife Research Center in Laurel, Maryland, is home to the world's largest collection of cranes. These cranes are used for research and for reintroduction programs. As of 20 October 2016, this collection included 77 of the highly endangered whooping cranes (*Grus americana*) used for reintroduction programs in Wisconsin and Louisiana.

The U.S. Fish and Wildlife Service was interested in protection of the endangered captive flock of whooping cranes through preventive vaccination, but with only approximately 500 of these birds in the world, including less than 150 in captivity, there was no possibility of doing safety and vaccination-challenge studies. Sandhill cranes (*G. canadensis*) were chosen as a suitable surrogate species for these needed vaccination-challenge studies. Similar use of sandhill cranes as surrogates for viral research of concern to whooping cranes has occurred with the Arbovirus that causes eastern equine encephalitis (Olsen et al. 1997, Olsen et al. 2005). A killed vaccine was used to produce immunity with eastern equine encephalitis (Olsen et al. 2005). No adverse reactions were encountered when vaccinating the cranes. No clinical signs of WNV disease were seen when the cranes were given the WNV challenge. Titer and necropsy results from the vaccination trials have been previously reported (Olsen et al. 2009), and a summary of the antibody titer results is presented in Table 1. We found a significant difference in titers between vaccinated and unvaccinated cranes at 14 days post-challenge ($P = 0.048$, $F = 5.44$) (Olsen et al. 2009). The objective of this paper is to summarize hematological responses to vaccination and challenge with WNV.

We selected adult sandhill cranes ($n = 12$) of mixed sexes that tested negative for previous exposure to WNV as measured by antibody titers. Seven of these cranes were vaccinated in the winter with 3 doses of 0.5-ml killed WNV vaccine (Fort Dodge Laboratories, Fort Dodge, IA; mention of commercial products does not imply U.S. Government endorsement) over a 4-week period at the USGS Patuxent Wildlife Research Center. Five sandhill cranes were injected with only sterile water (Table 1). Two months after completing the vaccinations, the sandhill cranes were shipped by commercial airline to the USGS National Wildlife Health Center, Madison, Wisconsin, where a BL-3 laboratory was available for the challenge phase of this study.

Following a 2-week adjustment period, the vaccinated ($n = 5$) and unvaccinated ($n = 5$) sandhill cranes were challenged by inoculating each with a 0.1-ml subcutaneous injection of a mosquito dose (5,000 plaque-forming units) of a WNV isolate from the original outbreak in New York State. Cranes designated as controls ($n = 2$) each received a 0.1-ml subcutaneous inoculation of sterile water. All cranes received health examinations, including taking 5.0-ml blood samples by jugular venipuncture for antibody titers and clinical pathology before, and at regularly scheduled intervals after inoculation with WNV. All cranes were humanely euthanized and necropsied at day 42 after challenge with the WNV.

Table 1. Titers of sandhill cranes inoculated with 1 mosquito dose (5,000 pfu [plaque-forming units]) of West Nile virus, USGS National Wildlife Health Center, Madison, Wisconsin, February 2002 (modified from Olsen et al. 2009).

Crane no.	Vaccinated	Challenged	Titers		
			Day 0	Day 14	Day 42
SC 003	Yes	No	<1:5	<1:5	<1:160[a]
SC 017	Yes	No	<1:5	<1:5	<1:10[a]
SC 001	Yes	Yes	<1:5	1:10240	1:10240
SC 028	Yes	Yes	<1:5	1:640	1:5120
SC 060	Yes	Yes	<1:5	1:2560	1:2560
SC 053	Yes	Yes	<1:5	1:10240	1:5120
SC 061	Yes	Yes	<1:5	1:20480	1:10240
SC 113	No	Yes	<1:5	1:320	1:2560
SC 004	No	Yes	<1:5	1:320	1:1280
SC 041	No	Yes	<1:5	1:1280	1:10240
SC 055	No	Yes	<1:5	1:640	1:2560
SC 065	No	Yes	<1:5	1:640	>1:2560

[a] Lowest titer tested.

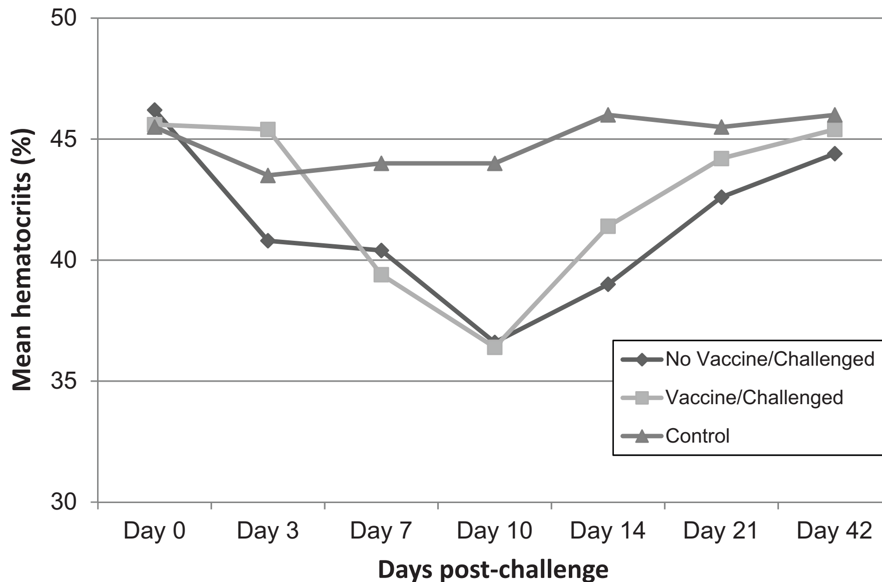

Figure 1. Mean hematocrits (%) of vaccinated/no challenge (control), vaccinated/challenged, and no vaccine/challenged sandhill cranes post challenge. Challenged whooping cranes were given 1 mosquito dose (5,000 pfu) of West Nile virus at the USGS National Wildlife Health Center, Madison, Wisconsin, February 2002.

Blood samples were collected by venipuncture of the right jugular vein (Dein 1984). Blood was placed in standard heparinized and plain blood tubes and blood smears made using the two coverslip method (Dein 1984). Blood smears were stained using Diff-Quick (American Scientific Products, mention of trade

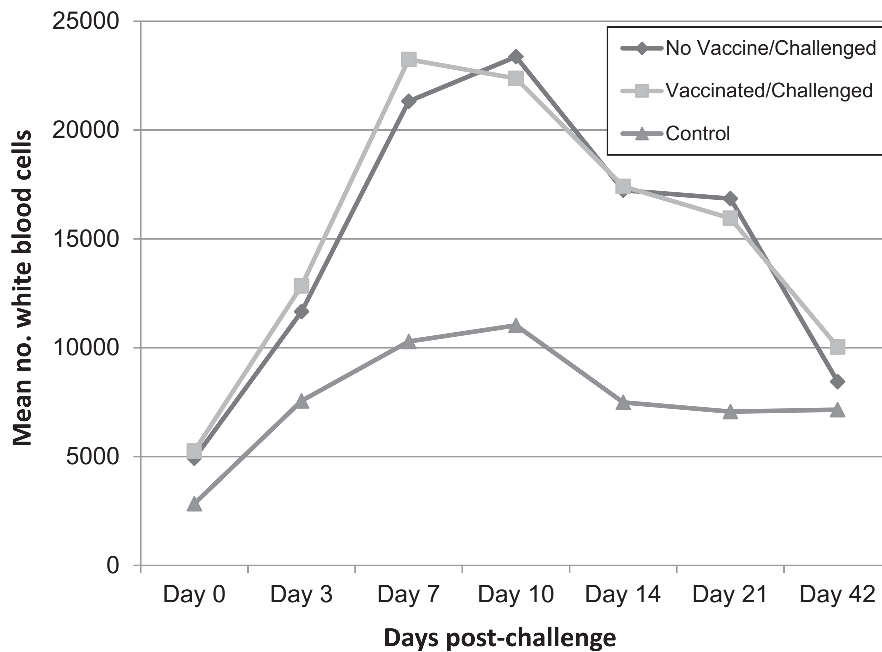

Figure 2. Mean white blood cell counts of vaccinated/no challenge (control), vaccinated/challenged, and no vaccine/challenged sandhill cranes. Sandhill cranes were challenged with 5,000 pfu of West Nile virus at the USGS National Wildlife Health Center, Madison, Wisconsin, February 2002.

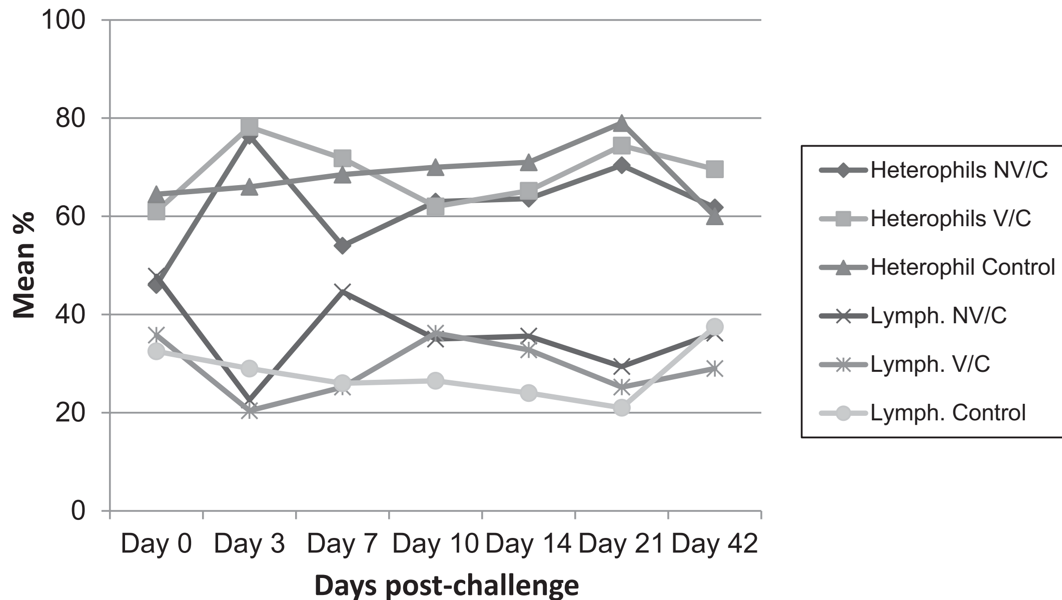

Figure 3. Mean percent heterophils and lymphocytes in vaccinated/no challenge (control), vaccinated/challenged (V/C), and no vaccine/challenged (NV/C) sandhill cranes. Sandhill cranes were injected (challenged) with 5,000 pfu of West Nile virus at the USGS National Wildlife Health Center, Madison, Wisconsin, February 2002.

name does not imply U.S. Government endorsement). Hematocrits were obtained by centrifuging a microhematocrit tube in a high speed centrifuge and reading the percent of red blood cells (Dein 1984). Total white blood cell counts were made by the Eosinophil Unopette Method (Dein 1984) (Becton-Dickinson, Test #5877) and then corrected for the percentage of heterophils and eosinophils in the differential count read from the blood smear (Dein 1984). This study was approved by the institutional animal care and use committees at the USGS Patuxent Wildlife Research Center and the USGS National Wildlife Health Center.

Between 1 and 2 weeks post-challenge, cranes exposed to the live WNV had lower hematocrits, whether or not they were previously vaccinated as compared to unchallenged controls (Figure 1). Mean white blood cell counts in all cranes given the live WNV challenge, whether vaccinated or not, were elevated as compared to the unchallenged controls (n = 2). The white blood cell count elevation lasted from day 3 to day 21 (Figure 2). Even though the white blood cell counts were elevated up to 2.5 times normal or control levels, there were no distinct shifts observed between heterophils and lymphocytes (Figure 3).

The most important findings were that vaccination of sandhill cranes with commercial killed equine WNV vaccine produced quickly elevating antibody titer levels when these previously vaccinated cranes were challenged by live WNV. Cranes with experimental infections with WNV had lowered hematocrits and elevated white blood cell counts as compared to control cranes not exposed to the virus housed under similar circumstances. This elevation in total white blood cell count occurred in both previously vaccinated cranes and unvaccinated cranes.

West Nile virus is a deadly virus for young cranes. In testing vaccines on adult sandhill cranes, we found that some blood parameters were altered by exposure to the virus. White blood cell counts were the most obvious and may be used as an indicator of WNV exposure in cranes, although this elevation in white blood cell counts is non-specific to WNV. Other hematology and serum chemistry results were studied and only hematocrit, percent heterophils, and percent lymphocytes were of interest, along with the already published information (Olsen et al. 2009) on titers encountered in experimental infections. Clinical pathology results showed challenged cranes, whether vaccinated or not, had a decrease in their hematocrits and an elevation of 2.5-fold in their white blood cell counts as compared to unchallenged control sandhill cranes. This is similar to a case report of a sandhill crane with an elevated white blood cell count found during a fatal WNV infection (Hansen et al. 2008). In this study no differences were apparent in the

differential counts of heterophils and lymphocytes. Our work would suggest that a combination of white blood cell counts and antibody titers can be used to diagnose and assess the severity of WNV infections in cranes.

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Key words: crane, Flavivirus, *Grus canadensis*, hematocrit, hematology, sandhill crane, West Nile virus, white blood cell count.