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WEST NILE VIRUS AND FERRUGINOUS HAWKS (*BUTEO REGALIS*) IN THE NORTHERN GREAT PLAINS—Emerging infectious diseases (EID) present significant threats to the conservation of global biodiversity (Daszak et al. 2000). Evaluating impacts (spatial, temporal and demographic) of EIDs on sensitive and declining wild-life populations is challenging because quantitative information is usually dependent on estimates rather than counts (Wobeser 2007) and mortality rates are seldom quantified with conventional monitoring (Naugle et al. 2005).

West Nile virus (WNV), a mosquito-borne *Flavivirus* (family *Flaviviridae*; Komar 2001), was discovered in north-west Uganda in 1937 (Smithburn et al. 1940). First encountered in the western hemisphere in New York in 1999 (Nash et al. 2001), WNV has become enzootic in all 48 contiguous states, and has been reported from 96% of counties to date (Centers for Disease Control and Prevention [CDC] 2013). West Nile virus has been detected in 65 species of mosquito in the United States (CDC 2012) and its ability to establish and persist across ecosystems and hosts is illustrated by its extensive distribution (CDC 2013). West Nile virus infection has been reported in 326 avian species (CDC 2013). Among a more scarcely occurring avian guild like the raptors, the CDC ArboNet Surveillance System (1999 to 2004) have documented infection in 36 species (Nemeth et al. 2006), approximately 70% of all raptor species occurring in North America.

In free-ranging raptors, WNV can be transmitted by a vector (e.g., mosquito), through oral exposure (infected food; after Root 2013), or direct contact with infected animals (e.g., in a nest; Komar et al. 2003). In *in situ* and controlled experiments, most passerines infected with WNV developed abnormal behavior, including neurologic signs such as lethargy, ataxia, disorientation, and weight loss (Komar et al. 2003). Clinical signs of adult raptors infected with WNV ranged from absent, mild and nonspecific, to acute and varied among species (Nemeth et al. 2006, 2008). Most adult raptors experimentally exposed to WNV were found to develop viremia within five days post-inoculation (Nemeth et al. 2006) and once seroconverted, WNV-induced humoral antibodies were found to persist throughout life (>4 years; Nemeth et al. 2008). Population and ecosystem-level impacts of WNV infection on declining populations of grassland obligate raptors (e.g., ferruginous hawk [*Buteo regalis*]) have not been documented.

In July 2013, while monitoring active ferruginous hawk nests in McPherson County, South Dakota (14T 5071049 N, 493014 W), we recovered five dead ferruginous hawk fledglings with estimated ages of 25 ($n = 3$) and 40 ($n = 2$) days from two tree nests. Remote camera footage indicated disorientation and ataxia in fledglings approximately three days prior to death. Carcasses were submitted to the Animal Disease Research and Diagnostic Laboratory at South Dakota State University, Brookings, South Dakota, for examination.

All recovered carcasses underwent necropsy and microscopic examination of routine tissues by histopathology. All five birds had histologic evidence of subacute multifocal nonsuppurative encephalitis, with mononuclear cell cuffing around vessels (Fig. 1A) and diffuse nonsuppurative inflammatory infiltrates throughout the parenchyma. Occasional microglial gitter cells surrounded neuronal cell bodies. Immunohistochemistry (Smedley et al. 2007) revealed WNV antigen in numerous cerebral neurons (Fig. 1B). To confirm histologic findings, frozen brain samples were pooled within nests and submitted (National Veterinary Service Laboratory, Ames, IA) for reverse transcriptase polymerase chain reaction (RT-PCR; Shi 2001); WNV RNA was detected in both samples. Concurrent acute heterophilic myocarditis was present in younger fledglings ($n = 3$), while older fledglings ($n = 2$) from the second nest had concurrent lesions in multiple tissues related to *Escherichia coli* septicemia, as well as enteric coccidiosis. No other gross or histologic lesions were noted.

Old world data indicate that susceptibility to fatal infection with WNV varies markedly for birds, with high death rates in juveniles and incidence of circulating antibodies in adults (Work et al. 1955, Hull et al. 2006). Innate immunity is believed to play a role in protection against primary infection; however, humoral immunity is an essential component of immune control over primary infection and protection against subsequent infections (Diamond et al. 2003). Hahn et al. (2006) showed evidence of passive transfer of antibodies from seropositive females to nestlings; however, observed susceptibility of fledglings to WNV infection in the present scenario indicates a lack of adequate immune response (effective levels of neutralizing antibody) irrespective of the protective mechanism. Why clinical manifestation of WNV infection might occur in a small percentage of infected individuals is not well understood but has been attributed to host-dependent genetic factors like point mutation-dependent susceptibility (Mashimo et al. 2002). Ability of virus strains to modify virulence through mutation makes it even more capricious and difficult to control, rendering these already declining grassland populations highly vulnerable to infection and mortality. Antibody duration in WNV pre-exposed adult raptors might contribute to long-term survival and reproductive output (Nemeth et al. 2008) but fledgling susceptibility to WNV infection is detrimental to recruitment and nesting success.

Ferruginous hawks are a grassland/shrubland obligate nesting raptor (Woffinden and Murphy 1989) and prefer to nest in lightly grazed pasture or idle areas (Lokemoen and Duebber 1976, Blair 1978, Blair and Schitoskey 1982). In the Great Plains, breeding range, local abundance, and productivity of several populations of ferruginous hawk have declined (Woffinden and Murphy 1989, Houston and Schmutz 1999); they have vacated close to half of their reproductive range on the northern prairies (Houston and Bechard 1984, Schmutz 1984, Houston and Schmutz 1999). Within the

northern prairie, landscapes with $\geq 50\%$ agricultural crop production are only marginally suitable for ferruginous hawks (Schmutz 1987, 1989), which has been attributed to the availability of Sciurid prey (Schmutz 1989, Zelenak and Rotella 1997). Impacts of anthropogenic habitat modifications and reduced prey availability on ferruginous hawks are compounded by WNV-caused mortality; an EID that demands coordinated long-term surveillance, monitoring, and research examining the prevalence and ecology of the virus among reservoir hosts (Naugle et al. 2005). Though susceptibility and reservoir competence decreases with increasing accumulation of adult survivors of WNV infection in a population (Nemeth et al. 2008), evidence and understanding of passive transfer of protective antibodies to nestlings is limited.

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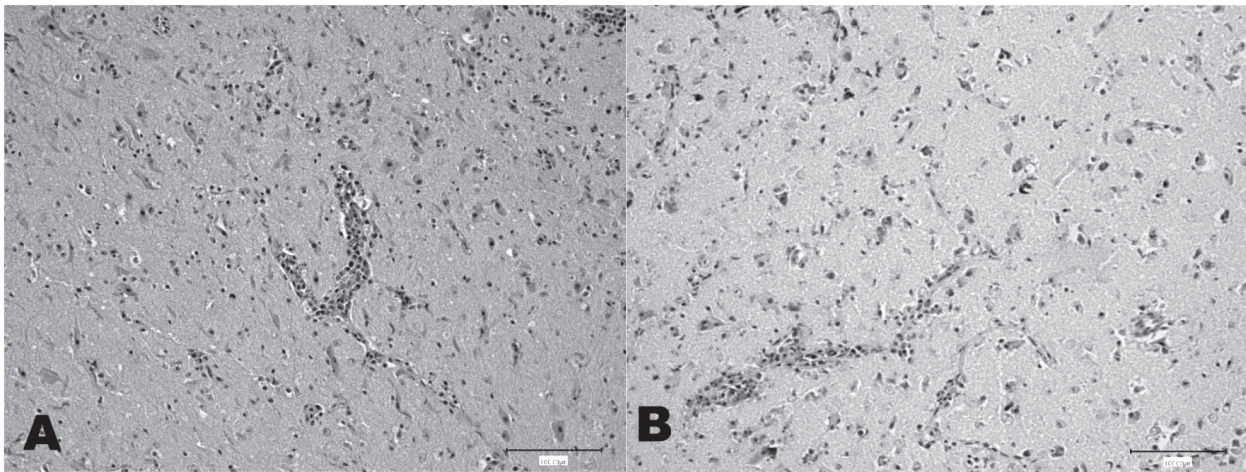


Figure 1. Photomicrographs of brain tissue from ferruginous hawk fledgling with West Nile virus infection. A) Cerebrum, Mononuclear cell cuffing around intracerebral vessels and diffuse nonsuppurative encephalitis HE. Bar = 100 µm, and B) Cerebrum, Immunohistochemical demonstration of WNV antigen within cerebral neurons. Toluidine blue. Bar = 100 µm.