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Eastern Equine Encephalitis Virus in Visibly Affected Ruffed Grouse (*Bonasa umbellus*) in Michigan, Minnesota, and Wisconsin, USA

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ABSTRACT: Eastern equine encephalitis virus (EEEV) infects many avian species but has rarely been described in Ruffed Grouse (*Bonasa umbellus*). Between September and December 2019, 40 Ruffed Grouse, most in poor physical condition, were submitted to the Michigan, Wisconsin, and Minnesota (US) Departments of Natural Resources; eight were positive for EEEV.

Eastern equine encephalitis virus (EEEV) is an alphavirus with a wide range of naturally infected avian hosts including exotic game species, Wild Turkeys (*Meleagris gallopavo*), owls, Whooping Cranes (*Grus americana*), shorebirds, and a variety of passerines (Morris 1988). Clinical disease and ensuing mortality occur frequently in birds not native to North America (Fenner et al. 2011), including Chukar Partridges (*Alectoris chukar*; Lord and Calisher 1970) and Ring-necked Pheasants (*Phasianus colchicus*; Williams et al. 2000). Clinical manifestation in birds ranges from purely encephalitic to viscerotropic disease affecting multiple organ systems. In the eastern US, EEEV is maintained in enzootic bird-mosquito cycles in freshwater wetlands. Some mosquitoes, especially *Culiseta melanura*, have an affinity for this environment, feed almost exclusively on birds, and act as the primary enzootic vector of EEEV (Fenner et al. 2011). Rainfall and other climatic and biologic factors (Letson et al. 1993) can contribute to increases of circulating virus in avian hosts. Under these conditions, mosquito species with wider host and geographic ranges, known as bridge vectors, have a higher chance of transmitting the virus from birds to humans and other mammals outside wetland areas. In humans, EEEV can cause neurologic symptoms, severe lifelong sequelae, and death (Deresiewicz et al. 1997); thus its management and study are of great relevance to public health.

Because of the known mosquito-passerine cycle of this virus, it follows that EEEV has been identified for years in migratory passerines (Bigler et al. 1975) along migration routes (Lord and Calisher 1970). However, EEEV has also been found to infect native North American gamebirds; in 1957–58, 50% (n=52) of Ruffed Grouse (*Bonasa umbellus*) sampled in Wisconsin were EEEV-positive by virus neutralization. In the same study, EEEV antibodies were found in Sharp-tailed Grouse (*Tympanuchus phasianellus*), Northern Bobwhite (*Colinus virginianus*), and many passerines; all the samples in that survey were taken from road kill or hunter-harvested birds, none of which were noted to be sick-looking (Karstad et al. 1960). Eastern equine encephalitis virus was isolated from Northern Bobwhite sentinels used in detecting virus around Maryland game bird farms in 1965 (Lord and Calisher 1970) and in Northern Bobwhites housed next to sick exotic game birds in 1955–67 in Florida (Bigler et al. 1975); these bobwhites had serum antibodies to EEEV but no visible manifestations of disease. Few records exist of native game species becoming

TABLE 1. Convenience sampled Ruffed Grouse (*Bonasa umbellus*) carcasses tested for eastern equine encephalitis virus (EEEV) in Wisconsin, Minnesota, and Michigan, USA. All Ruffed Grouse were submitted, dead, to respective state Natural Resource Departments by citizens. Carcasses were tested at Michigan State University Veterinary Diagnostic Lab (MSU VDL; Michigan birds), Cornell University Animal Health Diagnostic Center (AHDC; Minnesota birds), and Wisconsin Veterinary Diagnostic Lab (WVDL; Wisconsin birds). For a bird to be considered EEEV positive at AHDC and MSU VDL, it must test positive on two PCR primer sets targeting different regions of the virus (E. Dubovi pers. comm.). At WVDL, a single probe is used to confirm positives with real-time PCR.

Bird ID	Date	State[a]	County	Sex[b]	Age[c]	Tissues for PCR	*n* Birds tested in state from September–December of year
18-1	15 September 2018	WI	Price	M	J	Brain	21
18-2	2 October 2018	WI	Ashland	F	A	Pooled (brain, heart)	21
18-3	2 October 2018	WI	Vilas	U	U	Brain	21
18-4	8 October 2018	WI	Price	M	U	Brain	21
18-5	16 October 2018	WI	Ashland	U	U	Brain	21
18-6	19 October 2018	WI	Oneida	U	U	Brain	21
18-7	20 October 2018	WI	Price	U	U	Heart	21
18-8	21 October 2018	WI	Douglas	F	A	Pooled (brain, heart)	21
19-1	28 September 2019	MN	Itasca	F	U	Heart	8
19-2	28 September 2019	MN	Itasca	F	U	Brain	8
19-3	29 September 2019	MN	Aitken	U	U	Brain	8
19-4	Late September 2019	MI	Ontanogan	F	A	Pooled (brain, heart, spleen, kidney, intestine)	14
19-5	14 October 2019	MI	Houghton	M	J	Pooled (brain, heart, spleen, kidney, intestine)	14
19-6	3 October 2019	MN	Itasca	F	J	Brain	8
19-7	7 October 2019	WI	Price	M	A	Pooled (brain, heart, kidney, spleen)	18
19-8[d]	27 October 2019	WI	Douglas	M	A	Pooled (brain, heart, kidney, spleen)	18

[a] WI = Wisconsin; MN = Minnesota; MI = Michigan.

[b] F = female; M = male; U = unknown.

[c] A = adult; J = juvenile; U = unknown.

[d] Most carcasses testing positive for EEEV were in poor body condition upon citizen collection and submission, whether they were hunter-harvested or found dead. Bird 19-8 was a road kill carcass that was, notably, in good body condition when found.

pathologically affected following naturally occurring EEEV infection and, overall, mortality of any native species is believed to be much less frequent than that seen in certain nonnative species (Morris 1988).

As part of routine disease monitoring of sick and dead birds, 14 citizen-submitted Ruffed Grouse carcasses were examined between September and December 2019 at the Michigan Department of Natural Resources (DNR), Wildlife Disease Laboratory (Lansing, Michigan, USA). Two of interest included a juvenile male (ID 19-5) and an adult female (19-4; see Table 1). Both carcasses were in poor physical condition with a prominent keel, atrophied breast musculature, and no visible fat deposits. Tissues were submitted to the Michigan State University Veterinary Diagnostic Laboratory (Lansing, Michigan); both grouse were positive for EEEV and negative for West Nile virus by PCR. In Wisconsin from September to December 2019, citizens submitted 18 Ruffed Grouse carcasses to the Wisconsin DNR (Madison, Wisconsin, USA) for necropsy; two of the 18 were positive for EEEV by PCR (birds 19-7 and 19-8) at the Wisconsin Veterinary Diagnostic Laboratory (Madison, Wisconsin). Of note, Wisconsin also had eight EEEV PCR-positive Ruffed Grouse (of 21 submitted carcasses, birds 18-1

through 18-8 in Table 1) in the fall of 2018. In fall 2019, eight Ruffed Grouse carcasses were submitted to the Minnesota DNR (St. Paul, Minnesota, USA); four birds were EEEV-positive (birds 19-1, 19-2, 19-3, 19-6) by PCR at the Animal Health Diagnostic Center (Ithaca, New York, USA).

Histologic lesions in birds with EEEV are typical of those seen in other avian viral infections. They include nonsuppurative encephalitis and vasculitis of the brain, characterized by pyknotic or karyorrhectic endothelial cells and sometimes perivascular lymphocytic cuffing (Williams et al. 2000). In heart tissue, lesions can resemble those of West Nile virus, namely hemorrhage, lymphoplasmacytic or histiocytic myocarditis, myocardial necrosis, and tissue mineralization or fibrosis (Gamino and Höfle 2013). Heart tissue of bird 19-4 contained scattered interstitial aggregates of lymphocytes, plasma cells, and histiocytes; this bird was diagnosed with mild multifocal lymphoplasmacytic interstitial myocarditis suggestive of viral disease. Bird 19-5 had extensive freeze-thaw artifacts that could have obscured lesions. Of the positive Minnesota grouse (19-1, 19-2, 19-3, 19-6), one showed focally extensive polioencephalitis and another showed perivascular nucleated cells within brain tissue. Both findings were consistent with a viral encephalitis, but freeze-thaw artifacts in all birds made definitive histologic diagnosis difficult. Analysis and interpretation of histopathology from Wisconsin grouse is ongoing, but six of the ten positive birds between 2018 and 2019 had microscopic lesions consistent with EEEV infection. Our study was limited by convenience sampling bias; predominantly thin birds were submitted and sampled because they are worrisome to hunters or citizens.

Eastern equine encephalitis was first detected in Michigan in horses (*Equus caballus*) after a large equine outbreak in 1942 (Brown 1947). Cases since then have occurred primarily in horses and humans in the southern Lower Peninsula. In 2005, an outbreak of eight visibly sick white-tailed deer represented the state's first EEEV-related mortality in wildlife (Schmitt et al. 2007). Our article represents the first documentation of visibly affected Ruffed Grouse in the Upper Midwest. The EEEV-positive Ruffed Grouse from Michigan were harvested in the Upper Peninsula, where there have historically been few cases of eastern equine encephalitis (Centers for Disease Control and Prevention, ArboNET 2020). All positive grouse from Minnesota and Wisconsin were collected or harvested in counties in the northern part of each state (Minnesota Department of Natural Resources 2019). Our findings indicate that Ruffed Grouse may become infected and visibly affected by EEEV more frequently than previously recognized. Furthermore, EEEV activity may have a more northernly geographic extent in the US, and its maintenance may not be confined to birds and mosquitoes in hardwood swamps. Increasing knowledge of how the virus overwinters and continuing to collect and test mosquitos and wildlife for EEEV remain important given the high mortality rate of EEEV-infected humans and horses (Fenner et al. 2011). Documenting disease in wildlife whose populations face numerous stressors is important for managing wild populations and monitoring arboviral disease risk for public health.

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