MONITORING WEST NILE VIRUS IN WISCONSIN'S RUFFED GROUSE POPULATION: YEAR TWO (2019) PRELIMINARY RESULTS

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This report examines the second year of preliminary results (2019) of a multi-year collaborative study monitoring West Nile virus in Wisconsin's ruffed grouse.

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Abstract

The second year of the Wisconsin Department of Natural Resources multi-year monitoring effort of West Nile virus (WNV) exposure in ruffed grouse (*Bonasa umbellus*) indicates that of 188 hunter-harvested samples tested, 37 (20%) had antibodies to WNV either confirmed (17 or 9%) or likely (20 or 11%), and viral genetic material was not found in any of the tested heart samples. Results from 2019 reveal that although ruffed grouse are exposed to WNV, they can develop antibodies to the virus and survive exposure. This study is designed to evaluate WNV exposure in hunter-harvested ruffed grouse over a multi-year period (2018–2020). Ruffed grouse exposure can vary annually based on a number of conditions including but not limited to: virus prevalence, availability of adept mosquito populations, and weather conditions. This monitoring effort will be unable to draw conclusions regarding morbidity and mortality of ruffed grouse due to WNV infections but will allow for a better understanding of the distribution, prevalence, and scope of WNV exposure in Wisconsin's ruffed grouse population from which additional actions can be pursued. Research involving survival analysis of free-ranging, ruffed grouse populations would be key to further evaluation of ruffed grouse response to WNV exposure.

Introduction

In 2017, data from roadside surveys suggested spring ruffed grouse (*Bonasa umbellus*) drumming activity increased statewide, and brood observations had also indicated an increase in production compared to 2016 results. Despite encouraging surveys that suggested a possibility for increased harvest opportunities for fall 2017, many grouse hunters reported fewer flushes than anticipated while afield. Similar reports had been conveyed from hunters and management agency staff in Michigan and Minnesota. Unexpected declines in drumming survey results in the spring of 2018 escalated concerns regarding recent trends in ruffed grouse numbers. Furthermore, concurrent research from Pennsylvania has suggested that when taken together with other supporting evidence, including habitat loss and forest maturation, the presence of West Nile virus (WNV) had coincided with declines in ruffed grouse abundance. Given these abrupt decreases in population trends and heightened concerns over perceived impacts of WNV on ruffed grouse, the Wisconsin Department of Natural Resources (WDNR) entered a collaborative disease monitoring project with the Michigan and Minnesota Departments of Natural Resources. This joint effort will provide a more comprehensive view of the prevalence of exposure of ruffed grouse to WNV across the Upper Great Lakes region than an individual state could execute alone and demonstrates the interest of regional biologists in responding to concerns of hunters and the public at large.

The primary objectives of this multi-year study are to:

1. Assess the feasibility of utilizing hunter-harvested ruffed grouse to obtain biological samples from harvested birds for disease screening and collecting relevant metadata.
2. Determine prevalence of exposure to WNV in ruffed grouse populations and if there is significant change by year in Wisconsin.
3. Evaluate if samples can be collected in sufficient numbers to assess prevalence across different regions of the state.
4. Examine submitted samples for evidence of clinical disease associated with WNV infection.

Methods

For each of the first two years of the monitoring project, the goal for Wisconsin was to distribute 500 self-sampling kits to ruffed grouse hunters, with priority being to collect samples from the primary grouse range in northern and central regions of Wisconsin. After successfully harvesting a grouse, hunters were asked to collect blood onto Nobuto blood filter strips, remove and collect the heart, collect and submit feathers to determine age and sex of the bird, and to complete a datasheet with other pertinent information including location of harvest and body condition of the grouse. Hunters were then asked to send all samples and the datasheet to the WDNR Science Operations Center in Madison.

Once received at the WDNR Science Operation Center, samples were processed and readied for shipment to the Southeastern Cooperative Wildlife Disease Study (SCWDS) to be tested for West Nile virus. Samples from each of the three participating states were sent to SCWDS to ensure consistent testing protocols. Once samples were received by SCWDS they underwent two different forms of testing. Blood samples were evaluated for the presence of antibodies to West Nile virus using a plaque reduction neutralization test. Heart samples were tested by reverse transcription polymerase chain reaction (rtPCR) for viral genetic material. Results were also analyzed by age and sex of the bird, location of harvest, and hunter-reported body condition.

Results

Wisconsin submitted and received results for 188 ruffed grouse samples from 25 counties across the northern and central regions of the state (Table 1). Thirty-seven (20%) samples had antibodies to WNV either confirmed (17 or 9%) or likely (20 or 11%; Fig. 1). The 20 “likely WNV” results were samples where virus could only be identified to the genus Flavivirus, which includes WNV among several other related viruses. The presence of antibodies indicates that the individual was exposed to WNV and developed an immune response to the virus. Viral genetic material was not found in any of the 188 heart samples (Fig. 2). Fragments of viral genetic material, if present, could indicate many things – such as that birds had fragments of the virus enter their system or been exposed to the virus recently, or that they had an ongoing exposure. Michigan submitted 247 samples and WNV antibodies were detected in 20 (8%) blood samples with exposure to the virus either confirmed (7 or 3%) or likely (13 or 6%); viral

genetic material was found in 1 heart sample. Minnesota submitted 317 samples and WNV antibodies were detected in 39 (12%) of the samples with exposure either confirmed (3 or 1%) or likely (36 or 11%). Viral genetic material was not found in any of the Minnesota heart samples.

Samples from Wisconsin were analyzed by sex (female, male, unknown) and age (adult, juvenile, unknown) to evaluate potential differences in WNV exposure. No differences were found in the presence of antibodies in blood samples between sexes (χ_4^2 = 1.49, P = 0.83), nor was there a difference between sexes regarding the presence of viral genetic material from the rtPCR test (χ_2^2 = 0.27, P = 0.88; Table 2). Likewise, no differences were found between age classes for presence of antibodies (χ_4^2 = 4.17, P = 0.38) or presence of viral genetic material (χ_2^2 = 1.86, P = 0.40; Table 2). Hunter-reported body condition (fat, normal, thin) was also evaluated, but no differences were found amongst condition levels for the presence of antibodies (χ_4^2 = 2.05, P = 0.73) or presence of viral genetic material (χ_2^2 = 0.51, P = 0.78; Table 3).

Eastern Equine Encephalitis virus (EEEV)

Two ruffed grouse heart samples from Wisconsin tested positive for Eastern equine encephalitis virus (EEEV; Fig. 2), a similar virus to WNV.

Eastern equine encephalitis virus can be found in states throughout the eastern portion of the U.S. It was first documented in Wisconsin's ruffed grouse population in the 1950s, when 50% of grouse sampled tested positive for antibodies to EEEV. Like WNV, testing has been limited and opportunistic in nature to this point. Given that the virus was first identified as present in the 1950s and likely still present through normal population cycles and historic highs in the 1970s–1990s, it is believed that EEEV may affect individual birds but likely does not influence grouse populations. Opportunistic sampling for EEEV in ruffed grouse is ongoing in Wisconsin.

Discussion

Findings thus far suggest that while ruffed grouse are exposed to WNV, individuals are capable of surviving exposure and developing an immune response. To date, this monitoring effort has provided important baseline data on prevalence of exposure to WNV in ruffed grouse across Wisconsin and the Upper Great Lakes region, something that until now has not been evaluated. Not only are there many factors that can affect WNV prevalence, but there are also many factors that can determine an individual's response to a viral exposure and the competency of their immune system. Further evaluation of morbidity and mortality associated with WNV on ruffed grouse should incorporate a full survival analysis that considers not only presence and absence of the virus but also key factors that may affect immune responses such as nutrition, available cover, weather-related impacts, etc.

There are potentially several reasons for the higher exposure rates that occurred in Wisconsin compared to our neighboring states (i.e., localized and variable levels of mosquito activity), and

we plan to examine these possibilities further after the multi-year sampling period has concluded.

Some have speculated that juvenile ruffed grouse or birds in poor condition may be more susceptible to WNV exposure. After two years of sampling, differences in WNV exposure among hunter-harvested ruffed grouse have not been detected by age-class or between males and females. Body condition would also not be expected to be a reliable indicator of WNV exposure – some ruffed grouse in seemingly excellent condition also tested positive for WNV exposure. It is pertinent to note that only two years of sampling has been completed, and analyses will be more robust after the final year of sampling has been finalized and trends can be assessed across the entire dataset.

Furthermore, it is important to remember the limitations of this current monitoring effort. Because samples are being taken from hunter-harvested ruffed grouse, and not from birds that are captured and radio-marked, like in an intensive demographic study, it is not possible to discern any population-level impact WNV may be having on ruffed grouse at this time. The current monitoring effort will be able to shed light on the prevalence and overall distribution of WNV exposure across Wisconsin and the Upper Great Lakes region.

Research has indicated that the best method for maintaining healthy ruffed grouse populations is to manage appropriate habitat. With higher quantities and quality of habitat, the more suited grouse will be to overcome environmental stressors; not only disease but also weather, predation and others.

For fall 2020, Wisconsin DNR will not be distributing any new sampling kits due to the ongoing COVID-19 pandemic, but the Department encourages any hunter(s) with unused kits from previous years to use them, as none of the contents within the kits expire, and submit for processing as in previous years. If a hunter has a kit that they will not be able to use, please consider giving the kit to a fellow hunter for use.

Acknowledgements

We are grateful to the Ruffed Grouse Society and Wisconsin Conservation Congress for their collaboration and assistance in distributing sampling kits. We greatly appreciate the grouse hunting community for taking time to provide the Department with samples from their harvested birds – without their support this study would not be possible.

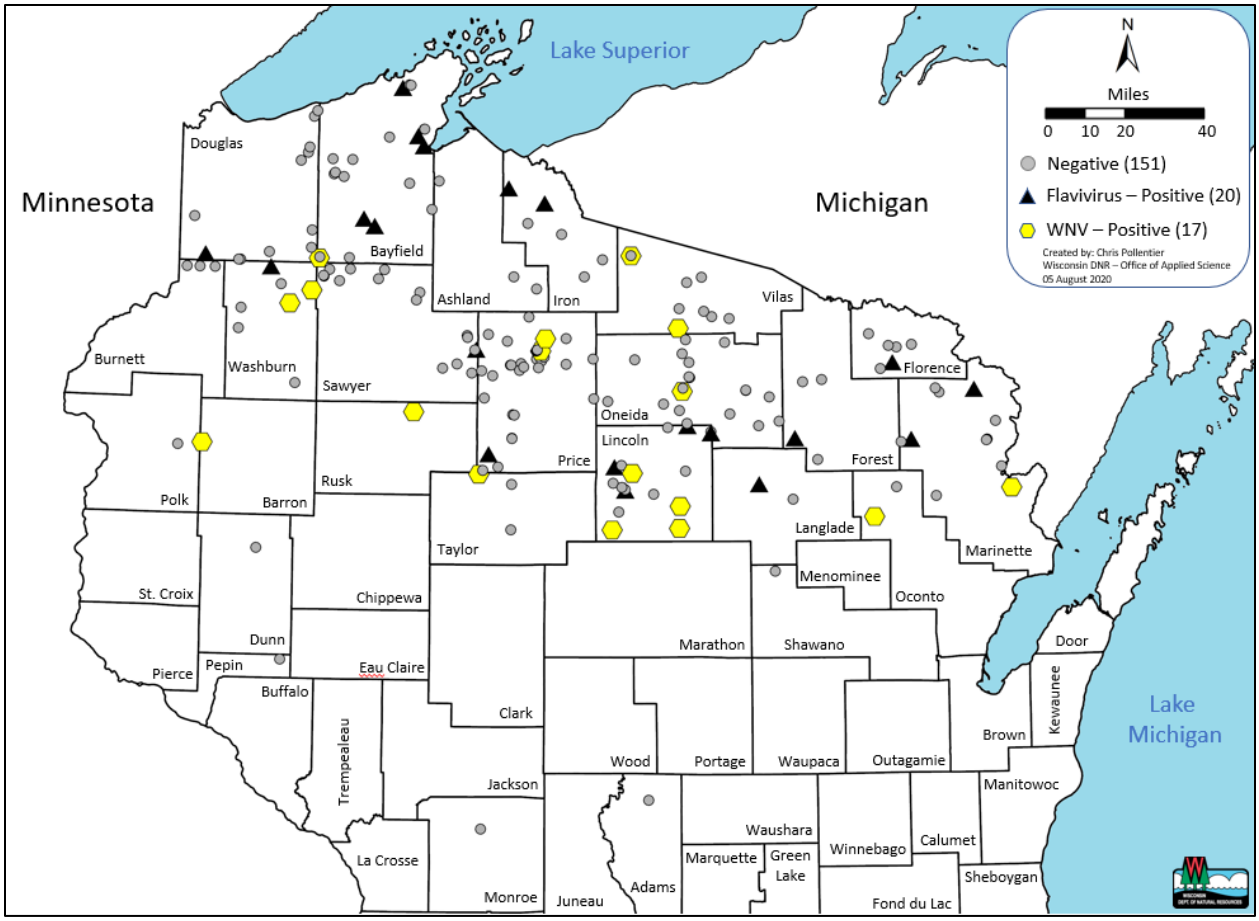

Figure 1. Distribution of hunter submitted ruffed grouse (*n* = 188) from the fall 2019 hunting season across Wisconsin, USA, tested for West Nile virus exposure. Serology tests examined the blood samples and results indicate whether ruffed grouse had confirmed West Nile virus exposure (*n* = 17, yellow dots) or likely exposure which were confirmed only to the genus level of Flavivirus (*n* = 20, black triangles). Flavivirus is a genus of viruses that includes West Nile virus among several other viruses. Negative test results (*n* = 151, gray dots) indicate those individual birds likely had not been exposed and antibodies to West Nile virus were not present.

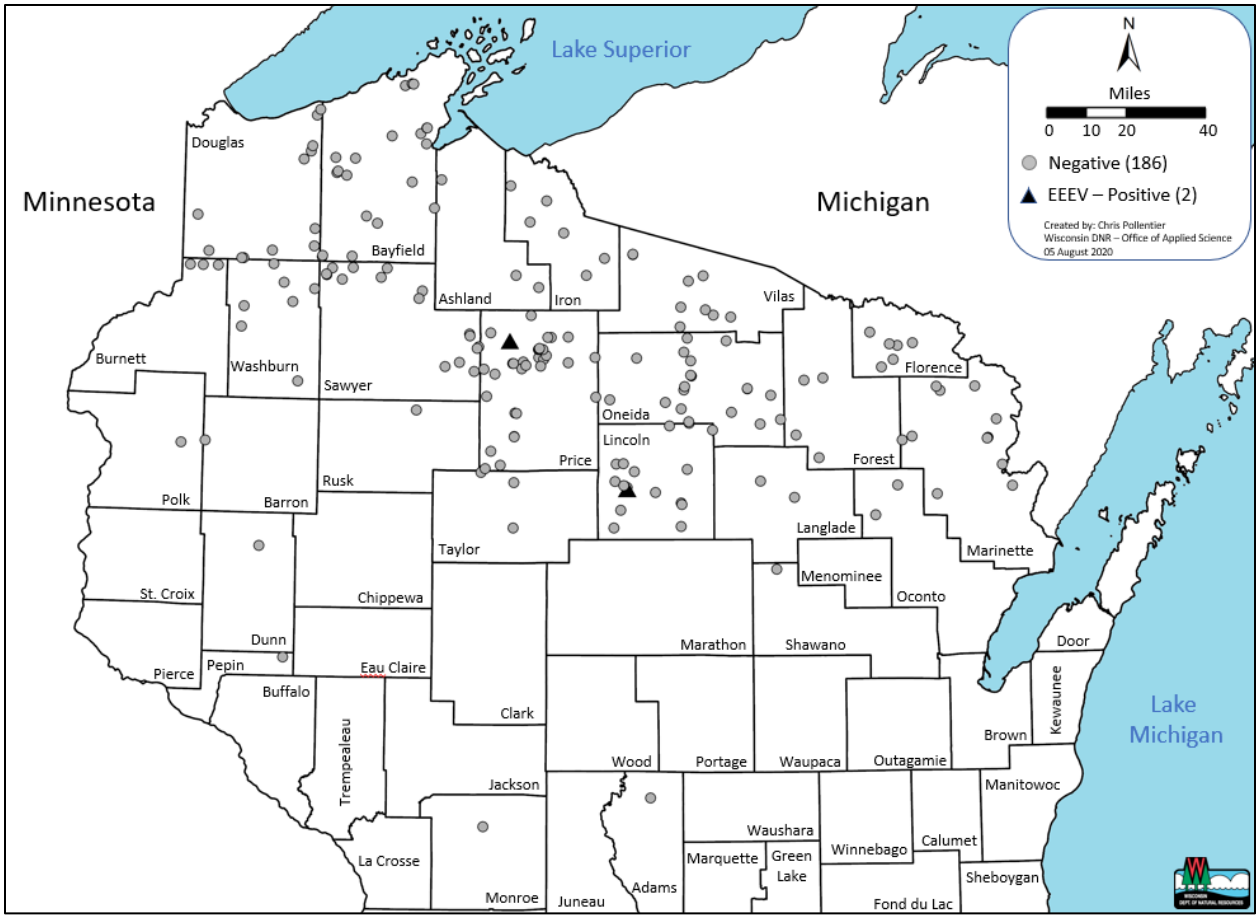

Figure 2. Distribution of hunter submitted ruffed grouse (*n* = 188) from the fall 2019 hunting season across Wisconsin, USA, tested for West Nile virus (WNV) exposure. Heart samples were tested for presence of WNV by reverse transcription polymerase chain reaction (rtPCR), and results indicate whether detectable fragments of viral genetic material were present in the heart samples. No samples tested positive for presence of WNV within heart tissues. For reference, a positive test would have suggested that those individual birds had recent exposure to WNV but does not directly indicate these birds were sick at time of harvest. Two harvested ruffed grouse, 1 each in Lincoln and Price Counties, respectively, showed positive test results for eastern equine encephalitis virus (EEEV, black triangles), which is also spread by the bite of a mosquito and is known to infect a wide range of animals including birds. Negative test results (*n* = 186, gray dots) indicate virus was not present in those individual birds.

Table 1. Summary of hunter-submitted ruffed grouse samples (*n* = 188) collected in 2019 from Wisconsin, USA, and tested for West Nile virus (WNV) exposure. Hunters submitted blood samples (serology) to test for presence of antibodies which indicated whether birds had been exposed to WNV, and the heart was tested for presence of WNV genetic material by reverse transcription polymerase chain reaction (rtPCR).

		Serology			rtPCR		
County	No. of samples	Negative	WNV	Flavivirus[a]	Negative	WNV	EEEV[b]
Adams	1	1	0	0	1	0	0
Ashland	3	3	0	0	3	0	0
Barron	1	0	1	0	1	0	0
Bayfield	20	14	1	5	20	0	0
Burnett	3	3	0	0	3	0	0
Douglas	12	11	0	1	12	0	0
Dunn	1	1	0	0	1	0	0
Florence	6	5	0	1	6	0	0
Forest	4	3	0	1	4	0	0
Iron	6	4	0	2	6	0	0
Langlade	2	1	0	1	2	0	0
Lincoln	17	11	3	3	16	0	1
Marinette	12	9	1	2	12	0	0
Monroe	1	1	0	0	1	0	0
Oconto	2	1	1	0	2	0	0
Oneida	21	19	1	1	21	0	0
Pepin	1	1	0	0	1	0	0
Polk	1	1	0	0	1	0	0
Price	34	31	2	1	33	0	1
Rusk	1	0	1	0	1	0	0
Sawyer	17	16	0	1	17	0	0
Shawano	1	1	0	0	1	0	0
Taylor	4	3	1	0	4	0	0
Vilas	9	7	2	0	9	1	0
Washburn	7	4	2	1	7	0	2
Unknown[c]	1	0	1	0	1	0	0
Totals	188	151	17	20	186	0	2

[a] Flavivirus is a genus of viruses that includes West Nile virus. During lab testing, the antibodies present in some blood samples were consistent with flavivirus and are likely to be West Nile virus (though not confirmed).

[b] During lab testing, 3 ruffed grouse heart samples tested positive for eastern equine encephalitis virus (EEEV), which is also spread by the bite of a mosquito and is known to infect a wide range of animals including birds.

[c] County or location of harvest was not provided on the returned datasheet.

Table 2. Sex and age of hunter-submitted ruffed grouse samples (*n* = 188) collected in 2019 from Wisconsin, USA, and tested for West Nile virus (WNV) exposure. Hunters submitted blood samples (serology) to test for presence of antibodies which indicated whether birds had been exposed to WNV, and the heart was tested for presence of WNV genetic material by reverse transcription polymerase chain reaction (rtPCR).

	Serology			rtPCR		
Parameter	Negative	WNV	Flavivirus[a]	Negative	WNV	EEEV[b]
Sex						
Female	58	9	8	74	0	1
Male	76	7	10	92	0	1
Unknown sex	17	1	2	20	0	0
Age						
Adult	75	12	11	96	0	2
Juvenile	66	4	9	79	0	0
Unknown age	10	1	0	11	0	0

[a] Flavivirus is a genus of viruses that includes West Nile virus. During lab testing, the antibodies present in some blood samples were consistent with flavivirus and are likely to be West Nile virus (though not confirmed).

[b] During lab testing, 2 ruffed grouse heart samples tested positive for eastern equine encephalitis virus (EEEV), which is also spread by the bite of a mosquito and is known to infect a wide range of animals including birds.

Table 3. Body condition of hunter-submitted ruffed grouse samples (*n* = 188) collected in 2019 from Wisconsin, USA, and tested for West Nile virus (WNV) exposure. Body condition was evaluated by hunters after successfully harvesting a ruffed grouse and was determined by the overall size of the breast muscles and whether the keel was visible. Hunters submitted blood samples (serology) to test for presence of antibodies which indicated whether birds had been exposed to WNV, and the heart was tested for presence of WNV genetic material by reverse transcription polymerase chain reaction (rtPCR).

	Serology			rtPCR		
Body condition	Negative	WNV	Flavivirus[a]	Negative	WNV	EEEV[b]
Fat	25	1	3	29	0	0
Normal	116	13	15	142	0	2
Thin	6	1	0	7	0	0
Not indicated	4	2	2	8	0	0

[a] Flavivirus is a genus of viruses that includes West Nile virus. During lab testing, the antibodies present in some blood samples were consistent with flavivirus and are likely to be West Nile virus (though not confirmed).

[b] During lab testing, 2 ruffed grouse heart samples tested positive for eastern equine encephalitis virus (EEEV), which is also spread by the bite of a mosquito and is known to infect a wide range of animals including birds.