WEST NILE VIRUS EXPOSURE AND INFECTION RATES IN MINNESOTA RUFFED GROUSE

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SUMMARY OF FINDINGS

Cooperating hunters (n = 117) voluntarily collected 273 samples from ruffed grouse (*Bonasa umbellus*) harvested during the 2018 hunting season as part of a multi-state, collaborative West Nile virus (WNV) study. Hunters collected biological samples (blood and heart) and information on the age, sex, and location where the bird was harvested. Blood and heart samples were submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia to assess both exposure to the virus and if the bird was undergoing an active infection. Laboratory results will be returned summer 2019 and will be shared with participating hunters. These findings will further understanding of the role that WNV plays in ruffed grouse in Minnesota and allow comparisons with other states in the Great Lakes Region.

INTRODUCTION

West Nile virus (WNV) is a mosquito-borne virus that causes encephalitis and myocarditis in individuals with active infections. West Nile virus has historically been found in Europe and Africa, but was first detected in North America in 1999 when an outbreak of encephalitis was reported in humans in New York City (Eidson et al 2001). Since its arrival, WNV has become established in all of the lower 48 US states and has been reported in over 300 bird species (Center for Disease Control, 2017), including ruffed grouse. Although some species, like American crows (*Corvus brachyrhynchos*) and blue jays (*Cyanocitta cristata*), readily die of WNV infection; most infected birds survive. Interestingly, since the arrival of WNV to the United States, mortality events due to WNV in some bird species have never been documented, [e.g. American robins (*Turdus migratorius*), chickadees (*Poecile spp.*), house wrens (*Troglodytes aedon*)] whereas others had an initial period of reduced survival for several years until they gained immunity to the virus, and yet some still continue to die from WNV annually (LaDeau et al. 2007, George et al. 2015).

The first documented WNV case in ruffed grouse in Minnesota occurred in 2005 (Ruffed Grouse Society, unpublished data). The suspected mosquito vector for ruffed grouse in Minnesota, *Culex restuans,* feeds almost exclusively on birds and is abundant in forests (D. Neitzel, Minnesota Department of Health, personal communication). We suspect that ruffed grouse in northern Minnesota are exposed to WNV annually but do not know if the virus causes active infections in juvenile or adult birds. Experimental infection of ruffed grouse and sage grouse (*Centrocercus urophasianus*) with WNV has indicated high susceptibility of these species to the disease (Naugle et al. 2004, Clark et al. 2006, Nemeth et al. 2017). Furthermore, recent study of the potential impact of WNV on ruffed grouse suggested chick survival was negatively affected by the virus (Nemeth et al. 2017). Past avian WNV outbreaks have occurred at the beginning of summer (late-June through mid-July; George et al. 2015) when grouse chicks may be most vulnerable to mortality.

Concern for WNV in ruffed grouse in Minnesota was heightened after the 2017 hunting season failed to meet harvest expectations, following a spring drumming count increase of 57% from

the previous spring (Roy 2017). Hunters expected better-than-average hunting experiences, as has historically been the case when the 10-year cycle is nearing its peak (Amman and Ryel 1963, Stoll 1980). However, the drumming count is an index to the adult breeding population and these surveys occur before annual production, meaning drumming counts do not necessarily forecast the juvenile contribution to the fall population. Poor grouse production can adversely impact hunter experiences because juveniles comprise much of the fall harvest (Dorney 1963). Despite 10-year cycles around a stable population average for decades in the core of Minnesota ruffed grouse range, some hunters indicated that hunting experiences have been less rewarding over that time period, leading many to speculate that something has been affecting juvenile production.

In an effort to understand the effects of WNV on ruffed grouse populations, Pennsylvania researchers conducted statewide serosurveys for WNV from hunter-harvested birds in 2016 and 2017 and found apparent prevalence rates of 14% (n = 202) and 22% (n = 217), respectively (J. Brown, Pennsylvania Game Commission, unpublished data). A recent study in Pennsylvania indicated that ruffed grouse population recovery may be impaired in areas with poorer habitat and WNV (Stauffer et al. 2018). The interaction between invasive diseases and land-use can result in complex effects on survival of wild birds (George et al. 2015). In 2017, the Michigan Department of Natural Resources (MIDNR) also confirmed WNV in wild ruffed grouse in Michigan for the first time. Two grouse were found dead, and 3 hunter-harvested grouse were submitted for testing because they were malnourished and acting strangely; heart lesions caused by WNV were observed in all 5 cases (MIDNR, 2017). Recently, WNV was also confirmed for the first time in Wisconsin ruffed grouse, as 3 birds tested positive for the virus, with 2 also being co-infected with Eastern equine encephalitis (Wisconsin DNR, unpublished data).

In this pilot study we are assessing WNV exposure and infection rates in Minnesota ruffed grouse during the fall by partnering with grouse hunters to obtain samples from their harvested birds. We are estimating serological exposure to WNV and also examining hearts for lesions consistent with the disease in both juvenile and adult grouse. Juvenile birds may represent recent population exposure to WNV, for a direct correlation to current viral load on the landscape; whereas adult birds represent either recent or maintained exposure, given the magnitude of their titer levels and presence or absence of associated lesions.

Importantly, this study is a multi-state collaborative effort with other natural resource agencies in the Great Lakes Region, including Wisconsin and Michigan. This concerted effort will provide a more comprehensive view of the role of WNV in the region than any individual state could execute alone and demonstrates the interest of regional biologists in responding to hunter concerns.

OBJECTIVES

1. Assess the feasibility of working with grouse hunters to obtain biological samples from wild ruffed grouse for disease screening and to collect relevant metadata.
2. Estimate exposure to WNV in ruffed grouse populations in northwest and north-central MN.
3. Determine prevalence of active infections of WNV in ruffed grouse populations in northwest and north-central MN by age class (juvenile and adult).
4. Correlate exposure to WNV with active infection using paired samples from the same bird.

METHODS

Our study area focused on a 60-mile radius around Grand Rapids, Longville, and Bemidji, MN (Figure 1), with a sample goal of 400 birds during the fall 2018 hunting season. This area was chosen in an attempt to sample along a moisture gradient from west to east, based on rainfall

received the previous year, and to simplify logistics of sampling kit dissemination to a few pick-up/drop-off locations. Regional MNDNR headquarters are located in Bemidji and Grand Rapids and provided a location for distribution of sampling kits. Pineridge Grouse Camp, which is located in Longville, was committed to assisting with our sampling effort and provided a third location for distribution of kits. Numerous organized hunts are also conducted annually in the study area (e.g., Ruffed Grouse Society National Hunt, Northwoods Bird Dogs/Bowen Lake Lodge, Akeley Grouse Hunt), which further facilitated sample collection.

Hunter Outreach

Multiple press releases were shared with the public with the first on 21 May 2018 announcing the multi-state collaboration between Wisconsin, Michigan, and Minnesota. Another press release came out on 23 Aug 2018 to provide more details for hunters interested in voluntarily participating in sampling efforts. Progress about the sampling efforts were shared by multiple media outlets throughout the hunting season to encourage public engagement (e.g., Duluth News Tribune, Outdoor News). We gave presentations at local universities (e.g., Itasca Community College, Bemidji State University), hunting camps (e.g., Pineridge Grouse Camp), regional DNR staff meetings, and distributed kits during these visits. We also attended organized hunts to distribute and collect kits (e.g., Akeley Grouse Hunt, Ruffed Grouse Society National Hunt, Northwoods Bird Dogs/Bowen Lake Lodge). A short paragraph about the study was added to the 2018 Minnesota Hunting Regulations and a contact was provided for more information. Information about the study was also posted on the Minnesota Department of Natural Resources website DNR Grouse Hunting Page.

Field Sample Collection

Each WNV sampling kit contained the following: 1 Nuboto filter strip (Advantec) for blood collection, 1 snack-sized zipper-top plastic bag (e.g., Ziploc brand) for storage of the filter strip in the field, 1 3-inch coin envelope for storage of filter strip once blood had dried, 1 4-oz whirlpak to collect the heart, a quart-sized zippered plastic bag for collection of feathers to confirm sex and age, and a datasheet to record hunter contact information and sampling location. A protocol was provided with each sampling kit along with *A Grouse in the Hand* pamphlets, courtesy of the Ruffed Grouse Society, to allow the hunter to determine the sex and age of their harvested bird based on feather characteristics. Ruffed grouse sex can be determined through tail length and rump feather dot patterns. Likewise, juvenile (<1 year) and adult (>1 year) age classes can be determined via fall feather wear of primary feathers collected from the wing. The instructions stressed the importance of collecting the blood on the filter strip within 30 min of harvest but also indicated samples collected after 30 min had value. Hunters were instructed to thoroughly coat the filter strip with blood until uniformly red and to allow the strip to air dry following the hunt. We asked hunters to record date and time of harvest and blood collection, location of harvest (GPS coordinates or distance and direction from nearest town), county of collection, hunter determined age class (juvenile, adult, or unsure) and sex (male, female, or unsure), any relevant comments, and hunter contact information (address, phone, and/or email address) if communication of results was desired. Samples collected through organized hunts or through local hunting camps were stored at room temperature (Nuboto strips) or frozen (heart samples) until submitted. Otherwise, hunters were provided with mailing kits with pre-paid UPS shipping labels, along with freezer packs and thermal bubble mailers to keep samples cold during shipment the following business day.

Laboratory Analysis

We confirmed age and sex of harvested birds before sending blood and heart samples to the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia (Athens, GA) for diagnostic testing after the end of hunting season. Sample results had not

been received from the lab at the writing of this report but are expected to arrive in summer 2019. Nuboto strips will be reconstituted to test for exposure to WNV, and viruses will be isolated. Hearts will be checked for virus by the polymerase chain reaction (PCR) and if virus is present, histological examination of the tissue will be performed.

Data Analysis

Apparent prevalence rates of WNV will be calculated using the number of positive detections from serum collected with Nuboto strips relative to the total number of blood samples collected. Seroprevalence by age class will be estimated by calculated apparent prevalence rates for juveniles and adults separately. Active infection rates will be calculated using the number of PCR-positive tests of heart tissue divided by the total number of heart samples submitted. Both seroprevalance and active infection data will be mapped using harvest location information and compared between the 2 sampling sites.

RESULTS AND DISCUSSION

Hunters (n = 117) collected 273 samples from ruffed grouse harvested during the hunting season 15 Sep 2018 – 1 Jan 2019 (Figure 2), of which 213 were collected from within the 60-mile sampling foci. Most of the samples (71%) were collected in October, 21% were collected in September, and submissions from November and December were 4.3% and 3.3%, respectively. Most of the returned kits contained all components requested, but 22 samples did not contain hearts, 40 samples were missing some or all feathers for sex or age determination, and 4 samples were missing location information.

Overall, the returned samples were from 160 juveniles (65%) and 87 adults (35%); however, age could not be confirmed for 26 birds due to missing primary feathers (Figure 3). The preponderance of juveniles in the sample was within the range reported by other studies (53% in Ohio, 75% in Wisconsin; Davis and Stoll 1973, Dorney 1963, respectively) and was expected given that juveniles typically make up the majority of birds harvested in the fall and in the fall population in general (Dorney and Kabat 1960, Dorney 1963). The sex of sampled birds was fairly evenly split between males (54%) and females (46%), but sex could not be confirmed for 14 birds due to missing feathers. Adult sex ratios for hunter harvested birds usually favor males, but juvenile sex ratios are usually closer to 50:50 (Dorney 1963, Davis and Stoll 1973). When our sample was split among age classes, males comprised 60% and females comprised 40% of the adult sample; whereas the sex ratio for juveniles was 50:50 as expected from other studies.

Verification of sex and age indicated that hunters were fairly accurate using feather characteristics for age and sex determination. However, the hunter-determined age needed to be corrected 51 of 212 times (24%), and sex was corrected 16 of 246 times (6.5%), not including cases where hunters indicated that they were unsure, or when feathers were not provided for verification.

We plan to share lab results with hunters about the birds they submitted and the overall findings of the study when we receive lab results. Given that we fell short of our sample goal by 32% for this pilot study, we will continue this study for 1 more year of data collection. We plan to expand the sampling area in fall 2019 to include a larger portion of ruffed grouse range in Minnesota and provide opportunities to other hunters interested in participating outside the original sampling area. To accomplish this, we will make sampling kits available at Wildlife Area Offices throughout ruffed grouse range in Minnesota. Sampling kits will be available on a first-come first-serve basis until depleted. We hope to collect 400 samples in the upcoming season to gain insights into year-to-year variability and inform regional comparisons.

ACKNOWLEDGMENTS

We would like to thank all the hunters that submitted samples for this study, with a special thanks to Pineridge Grouse Camp, Bowen Lodge and Northwoods Bird Dogs, the Akeley Grouse Hunt, the Ruffed Grouse Society, contributing students from Itasca Community College, DNR staff and retirees, as well as friends and family, with a special thanks to Charlie Tucker and Matt Breuer for their large sample contributions. We couldn't do this study without your help. We would also like to thank the Ruffed Grouse Society for providing partial funding of this study, as well as Federal Aid in Wildlife Restoration (Pittman_Robertson) for funding. We are grateful to Erik Hildebrand, Patrick Hagen, Margaret Dexter, and Lindsey Peterson for assistance with kit building. Mike Larson provided helpful comments on an early version of this report.

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Figure 1. The study area in 2018 was comprised of 60-mile radii centered on Bemidji, Grand Rapids, and Longville in Minnesota. The ruffed grouse drumming survey regions are indicated in blue for reference.

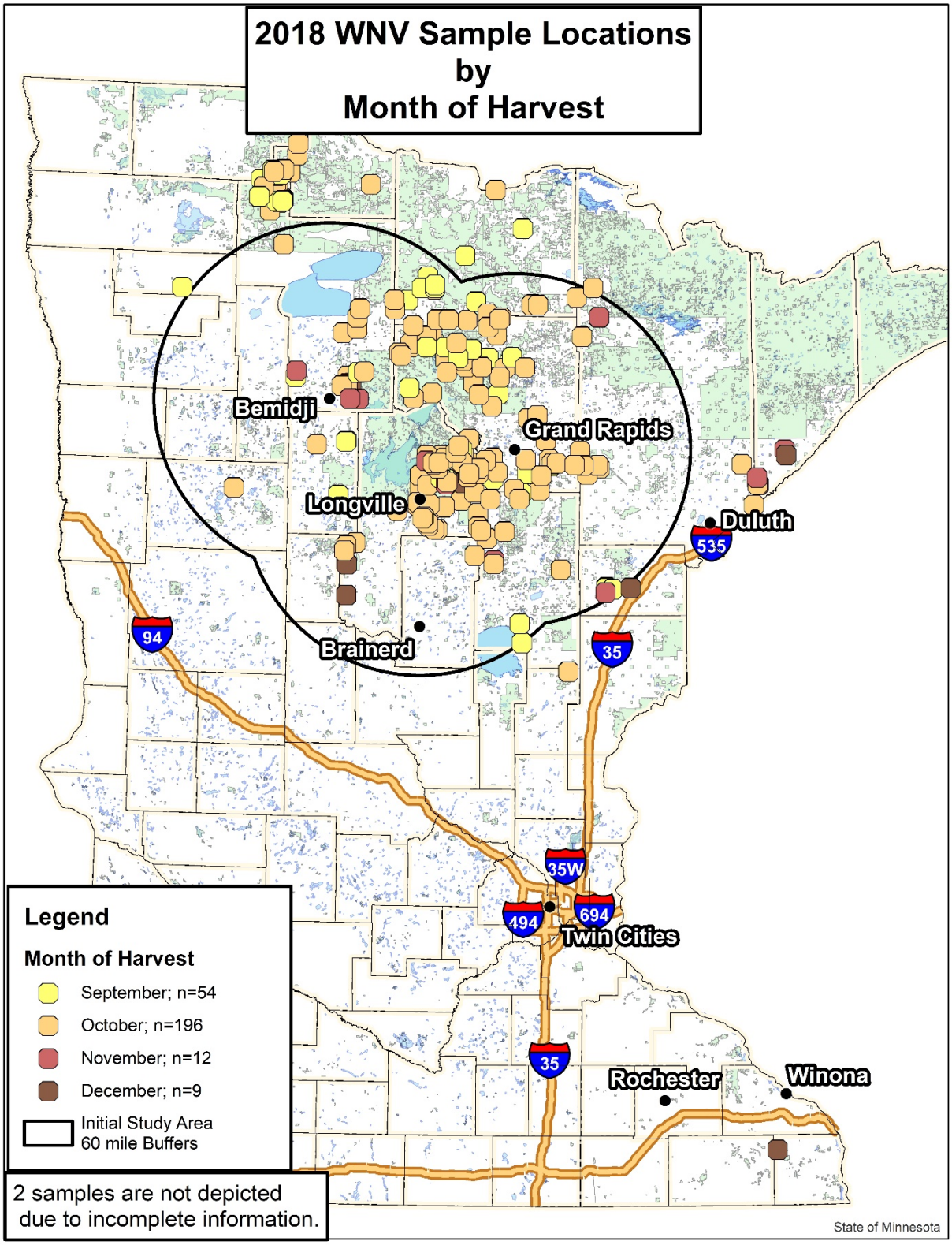

Figure 2. The distribution of hunter-harvested ruffed grouse samples both inside and outside the planned study area in Minnesota during hunting season in 2018.

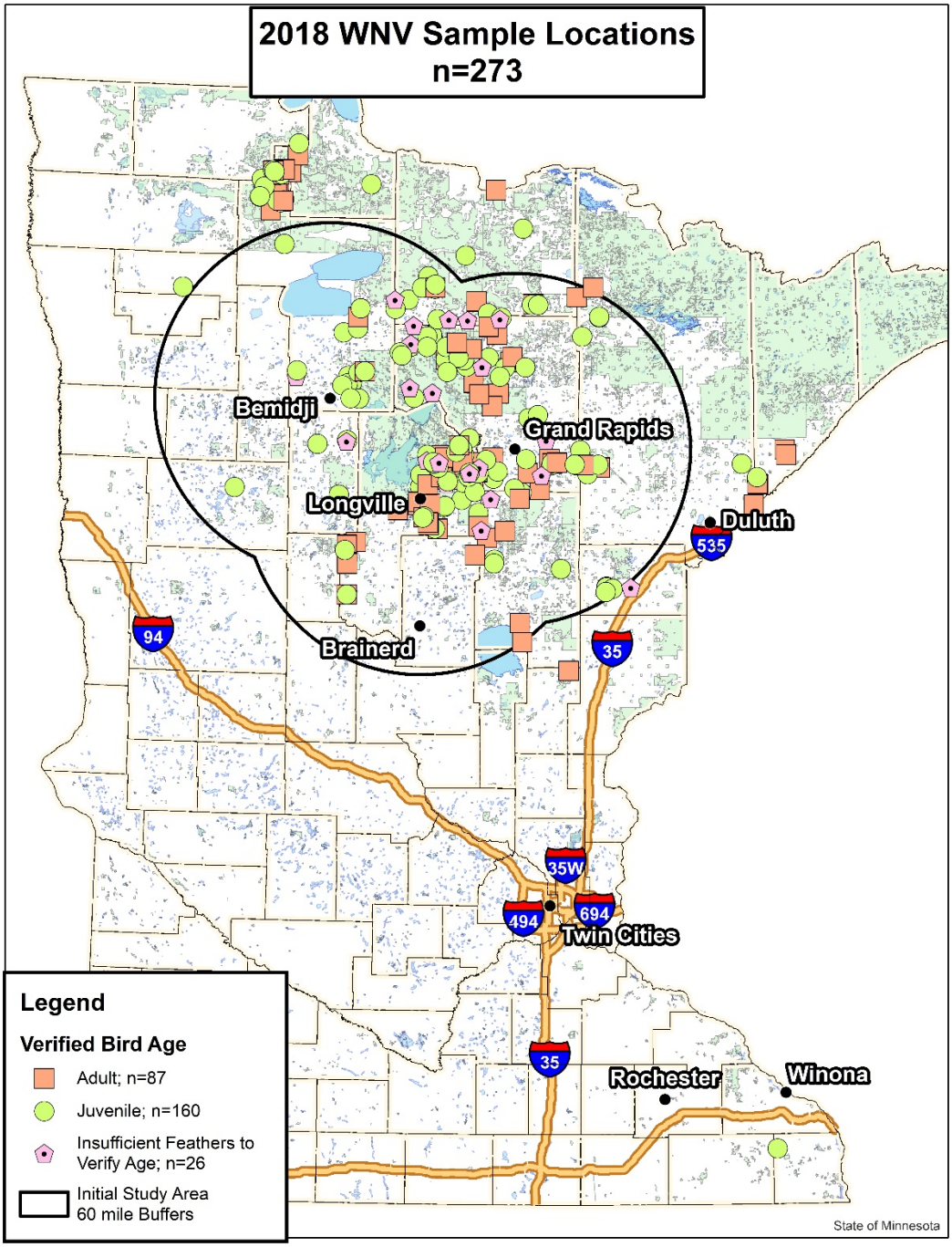

Figure 3. The age distribution of hunter-harvested ruffed grouse samples collected within and outside our study buffers in Minnesota during hunting season in 2018.