

West Nile Virus and Greater Sage-Grouse: Estimating Infection Rate in a Wild Bird Population

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1 **SUMMARY.** Understanding impacts of disease on wild bird populations requires knowing not only susceptibility to mortality following infection, but also the proportion of the population that is infected. Greater sage-grouse (*Centrocercus urophasianus*) in western North America are known to be extremely susceptible to mortality following infection with West Nile virus (WNV), but actual infection rates in wild populations remain unknown. We used rates of WNV-related mortality and seroprevalence from radiomarked females to estimate infection rates in a wild greater sage-grouse population in the Powder River basin (PRB) of Montana and Wyoming from 2003 to 2005. Minimum WNV-related mortality rates ranged from 2.4% to 13.3% among years and maximum possible rates ranged from 8.2% to 28.9%. All live-captured birds in 2003 and 2004 tested seronegative. In spring 2005 and spring 2006, 10.3% and 1.8% respectively, of newly captured females tested seropositive for neutralizing antibodies to WNV. These are the first documented cases of sage-grouse surviving infection with WNV. Low to moderate WNV-related mortality in summer followed by low seroprevalence the following spring in all years indicates that annual infection rates were between 4% and 29%. This suggests that most sage-grouse in the PRB have not yet been exposed and remain susceptible. Impacts of WNV in the PRB in the near future will likely depend more on annual variation in temperature and changes in vector distribution than on the spread of resistance. Until the epizootiology of WNV in sagebrush-steppe ecosystems is better understood, we suggest that management to reduce impacts of WNV focus on eliminating man-made water sources that support breeding mosquitoes known to vector the virus. Our findings also underscore problems with using seroprevalence as a surrogate for infection rate and for identifying competent hosts in highly susceptible species.

RESUMEN. El virus del oeste del Nilo y el gallo de las artemisas: Estimando la tasa de infección en una población de aves silvestres.

9 Para comprender el impacto de una enfermedad en poblaciones de aves silvestres se requiere conocer no solo la susceptibilidad a la mortalidad posterior a la infección, sino también la proporción de la población que se infecta. Se sabe que los gallos de las artemisas (*Centrocercus urophasianus*) en el oeste de los Estados Unidos, son extremadamente susceptibles a la mortalidad posterior a la infección con el virus del Oeste del Nilo, sin embargo, se desconoce la tasa de infección en animales silvestres. Durante los años 2003 al 2005, se utilizaron tasas de mortalidades relacionadas con el virus del oeste del Nilo y seroprevalencia en hembras marcadas, para estimar la tasa de infección en una población silvestre de gallos de las artemisas en la cuenca del río Powder en los estados de Montana y Wyoming, Estados Unidos. Durante los años evaluados, las tasas mínimas de mortalidad relacionadas con el virus del Oeste del Nilo estuvieron en un rango de entre 2.4% y 13.3% y las tasas máximas posibles en un rango de 8.2% a 28.9%. Los sueros de todas las aves vivas capturadas en el año 2003 y el año 2004 resultaron negativas. En la primavera de los años 2005 y 2006, el 10.3% y el 1.8%, respectivamente, de las hembras capturadas por primera vez resultaron serológicamente positivas para anticuerpos neutralizantes contra el virus de Oeste del Nilo. Estos son los primeros casos documentados de gallos de las artemisas sobrevivientes a la infección con virus del oeste del Nilo. Mortalidades relacionadas con el virus del oeste del Nilo de bajas a moderadas en el verano seguidas de baja seroprevalencia en la primavera siguiente durante todos los años, indican que las tasas anuales de infección estaban entre el 4% y el 29%. Esto sugiere que la mayoría de los gallos de las artemisas en la cuenca del río Powder no han sido expuestos al virus del oeste del Nilo y permanecen susceptibles. El impacto del virus del oeste del Nilo en el futuro cercano en la cuenca del río Powder, probablemente dependa más de variaciones anuales en la temperatura y cambios en la distribución del vector, que en la diseminación de resistencia. Hasta cuando entienda mejor la epizootiología del virus del oeste del Nilo en el ecosistema de las praderas, se sugiere que el manejo para reducir el impacto del virus del oeste del Nilo se concentre en la eliminación de fuentes de agua que sustentan la reproducción de mosquitos que son los vectores conocidos del virus. Nuestros hallazgos también enfatizan los problemas de la utilización de seroprevalencia como un método para sustituir la tasa de infección y para identificar huéspedes competentes en especies altamente susceptibles.

Key words: *Centrocercus urophasianus*, coal-bed natural gas, energy development, flavivirus, greater sage-grouse, infection rate, sagebrush-steppe, West Nile virus

Abbreviations: PRB = Powder River basin; PRNT = microplaque reduction neutralization test; WNV = West Nile virus

Assessing risks posed by emerging infectious disease is an increasingly important part of conservation planning and management for avian species of concern (6,9,20). Human modifications to wildlife habitat often facilitate the spread of infectious diseases (6,8,20), and disease outbreaks may undermine efforts to maintain viable or harvestable populations (21,26,35,39).

Predicting impacts of emerging infectious disease and identifying suitable strategies to control its spread requires knowing both disease prevalence and susceptibility of infected individuals to disease-related mortality (16,17). Susceptibility is typically estimated by experimentally infecting wild-caught animals in the laboratory (e.g., 4,16). Ideally, prevalence (i.e., exposure) would be measured by infection rate, defined as the proportion of the population that is exposed to the pathogen during an outbreak

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and becomes infected. Unfortunately, infection rates in wild populations are difficult to estimate (17,18,21). For that reason, most studies instead report seroprevalence as a surrogate for infection rate (e.g., 1,2,9,11,19,28,33). Drawing inferences regarding exposure based solely on seroprevalence assumes a linear relationship between the two. However, because seroprevalence estimates exclude infected individuals that die prior to sampling, seroprevalence may underestimate infection rate in susceptible species with high disease-related mortality (13,17). Estimates of host competence that rely on seroprevalence suffer from the same problem. If infected hosts die soon after transmitting the virus, measures of seroprevalence after the outbreak will underestimate true disease prevalence and the importance of that host in the transmission cycle. Thus, to fully understand the prevalence, impacts, and epizootiology of disease in wild bird populations requires that we estimate not only susceptibility and seroprevalence, but also disease-related mortality, which in turn allows estimation of actual infection rates.

Knowing infection rate is also crucial for identifying potential strategies for mitigating disease impacts to susceptible species. If infection rates are low, it suggests that exposure is uncommon, and that it may be possible to further reduce exposure by managing vectors or alternative hosts, or both. In contrast, if infection rates are uniformly high, then focusing on other management strategies, such as vaccination, may be more effective.

The recent spread of West Nile virus (WNV) in North America represents an important potential stressor on native bird populations, including greater sage-grouse (*Centrocercus urophasianus*) (hereafter "sage-grouse"). Sage-grouse are gallinaceous birds native to western semiarid sagebrush (*Artemisia* spp.) habitats (30). Previously widespread, the species has been extirpated over almost half of its original range because of loss, fragmentation, and degradation of sagebrush habitat (5,15,29). The species' conservation status has precipitated a coordinated effort to assess risks to populations and implement conservation and management actions to mitigate those risks (5). WNV was first detected in dead sage-grouse in 2002. By 2003, WNV-related mortality had reduced late-summer survival of adult females by 25% (22) and resulted in near-extirpation of a local breeding population in northeastern Wyoming (36). In summer 2004, survival was 10% lower (86%) at sites across the species' range with confirmed WNV mortalities than at sites without WNV mortalities (96%) (23). The extreme susceptibility of sage-grouse was confirmed in 2004 when, in separate laboratory trials, all nonvaccinated birds ($n = 44$) experimentally infected with WNV died within 6–8 days, regardless of dosage (4, T. Cornish, unpubl. data). As of fall 2006, sage-grouse mortalities positive for WNV have been confirmed in 11 of 13 states and provinces where the species still occurs (23,34). Despite concern over impacts of WNV on sage-grouse, actual prevalence of the virus in wild populations remains unknown.

Recent reviews of WNV have identified a lack of data on infection rates from wild populations as a major hindrance to understanding impacts of this recently arrived pathogen on North American birds (21,26). To better understand the prevalence and potential impacts of WNV on sage-grouse, we used rates of WNV-related mortality and seroprevalence from radiomarked females to retrospectively estimate annual WNV infection rates in a wild population from 2003 to 2005. We also examine implications of low infection rates for managing WNV risk in sage-grouse conservation and management strategies.

MATERIALS AND METHODS

Female sage-grouse were captured and radiomarked from 2003 to 2006 as part of a study assessing impacts of coal-bed natural gas development on sage-grouse populations in the Powder River basin (PRB) of southeastern Montana and northeastern Wyoming (elevation 1000–1400 m). Study sites primarily consisted of semi-arid sagebrush-steppe and shortgrass prairie interspersed with mesic shrubland, greasewood (*Sarcobatus vermiculatus*) bottomlands, irrigated and dryland crops, riparian woodland, and conifer forest. Dominant plant species in sagebrush-steppe included Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) and Plains silver sagebrush (*Artemisia cana cana*) with an understory of native and exotic grasses and forbs.

We monitored radiomarked females every 2–4 days during the peak WNV transmission period (July 1–September 15) in each year (36). Dead birds that yielded testable carcasses (i.e., carcasses with brain, wing or leg bones, internal organs, or spinal column present) underwent complete necropsies and microscopic examination of tissues by histopathology at the Wyoming State Veterinary Laboratory (Laramie, WY). Each carcass was tested for WNV using real-time polymerase chain reaction (31) and immunohistochemistry (14). Select cases positive for WNV were confirmed by isolation of the virus from one or more tissues (brain, heart, kidney, or bone marrow) in Vero cell cultures (32).

We used a Kaplan–Meier product limit estimator with staggered-entry design to estimate mortality in each year from 2003 to 2005 (38). Because 41% of 49 mortalities over the 3 yr did not yield testable carcasses, mortality estimates based only on carcasses that tested positive for WNV infection may have underestimated actual WNV-related mortality. For this reason, we calculated both a minimum WNV-related mortality rate based on mortalities confirmed positive for WNV and a maximum possible WNV-related mortality rate based on total mortalities minus those confirmed negative for WNV. The maximum possible estimate included mortalities for which no carcass (e.g., only a radiotransmitter) was recovered, those in which the carcass was not testable (e.g., too decomposed), and those in which tests were inconclusive. Individuals captured after July 1 were left-censored on the date of capture, and individuals that disappeared prior to September 15 (i.e., fate unknown) were right-censored on the last date they were located (38). We estimated dates of mortality as the midpoint between last date observed alive and the first date observed dead. In some cases, we estimated timing of mortality more accurately from the condition of the carcass.

To determine whether sage-grouse survived infection with the virus, we collected blood samples from live-captured birds in August–September 2003 ($n = 55$), March–April 2004 ($n = 66$), August–October 2004 ($n = 46$), March–April 2005 ($n = 58$), and March–April 2006 ($n = 109$). Blood samples were refrigerated and centrifuged, and serum was decanted within 12 hr of capture, then frozen until testing. Serum samples were tested for neutralizing antibodies to WNV using a microplaque reduction neutralization test (PRNT) (37). All samples positive for WNV were also tested for St. Louis encephalitis virus, the only other flavivirus in this region known to cross-react serologically with WNV (3). We report seroprevalence as the proportion of females that tested positive (PRNT titer 1:100) for antibodies to WNV and calculated 95% confidence intervals using logistic regression.

We used rates of WNV-related mortality during the WNV season and observed seroprevalence the following spring to calculate infection rates in each year from 2003 to 2005. We based our calculations on the mathematical model of Komar *et al.* (17), who

2 showed that infection rate, I, can be expressed in terms of postepizootic seroprevalence, S, and susceptibility to mortality following WNV infection, M, as follows:

$$I = S / [1 - M - (S * M)] \quad (1)$$

Because susceptibility, M, can be expressed as WNV-related mortality, ΔP, divided by infection rate (i.e., $M = \Delta P / I$), substituting for M and rearranging the equation allowed us to calculate infection rate from WNV-related mortality rate and postepizootic seroprevalence:

$$I = \Delta P + S - (S * \Delta P) \quad (2)$$

Because WNV-related mortality reduces post-epizootic population size and inflates the seroprevalence estimate, the $S \Delta P$ term in Eq. 2 is used to adjust the postepizootic seroprevalence estimate to reflect seroprevalence based on population size prior to, rather than following, the outbreak. Our method assumes the following: 1) that additional WNV-related mortality did not occur between the end of the WNV transmission period and when serum samples were collected the following spring and 2) that birds seropositive in spring were infected the previous summer.

To assess the accuracy of seroprevalence as a measure of actual infection rates, we also examined the relationship between infection rate and postepizootic seroprevalence over a range of observed susceptibilities across species (4,16).

RESULTS

Estimated minimum WNV-related mortality was low in all years, ranging from 2.4% in 2005 to 13.3% in 2003 (Table 1). Maximum possible WNV-related mortality was low to moderate, ranging from 8.2% to 28.9% (Table 1). WNV-related mortality rates were significantly higher in 2003, the sixth warmest summer on record in the PRB, than in 2004 and 2005, the 86th and 41st warmest, respectively (24).

All serum samples through fall 2004 ($n = 167$) tested negative for WNV. In spring 2005, six of 58 (10.3%) females captured tested seropositive. In spring 2006, two of 109 (1.8%) females tested seropositive. All females seropositive for WNV tested negative for St. Louis encephalitis virus (PRNT titers $< 1:10$). Of the six seropositive females in 2005, four were yearlings (i.e., hatched in 2004) and two were adults (i.e., hatched in 2003 or earlier). Of the two seropositive females in 2006, one was a yearling (i.e., hatched in 2005), and one was an adult (i.e., hatched in 2004 or earlier). All birds were of normal mass for their age (mean \pm SE; adult: 1.64 ± 0.05 kg, $n = 3$; yearling: 1.44 ± 0.04 kg, $n = 5$) and exhibited no overt signs of WNV-related disease at capture (e.g., morbidity, ataxia, tilted head, drooping wings, or impaired flight) (4,18,36). Seropositive females also initiated nests at the same time as other hens and had normal clutch sizes. The presence of neutralizing antibodies to WNV in yearlings captured in spring indicates that antibodies were detectable for at least 5 mo following exposure. Seropositive females were distributed across six leks at four different study sites. Microsatellite analyses demonstrated that none of the eight females were related and thus can be considered independent replicates for seroprevalence calculations (K. L. Bush, University of Alberta, unpubl. data).

Estimates of both minimum and maximum possible infection rates in the PRB were low to moderate in all 3 yr (Table 1). Estimates of minimum infection rate ranged from 4.2% to 13.6% and maximum possible infection rate ranged from 9.9% to 28.9%. Infection rates were higher in 2003 than in 2004 or 2005. Sample

Table 1. Mortality and WNV testing of radiomarked female sage-grouse mortality during the peak WNV transmission period (July 1–September 15) and seroprevalence the following spring in the PRB, 2003–2005. Estimated minimum and maximum mortality, seroprevalence, and minimum and maximum infection rates given as mean (95% CI).

Year	No. study sites ^A	No. females monitored	No. mortalities	No. tested for WNV	No. positive/negative tests	Median date of WNV-related mortality	Minimum WNV-related mortality (%)	Maximum possible WNV-related mortality (%)	Seroprevalence (following exposure)	Minimum infection rate	Maximum infection rate
2003	3	46	15	10	6/4	Aug. 3 (Jul. 24–Aug. 5)	0.133 (0.048–0.219)	0.289 ^B (0.178–0.399)	0.000 (–)	0.135 ^B (0.048–0.219)	0.289 ^B (0.178–0.399)
2004	6	118	17	9	4/5	Aug. 23 (Aug. 3–Sept. 4)	0.037 (0.003–0.071)	0.094 (0.042–0.147)	0.103 (0.047–0.212)	0.136 (0.070–0.202)	0.187 (0.011–0.265)
2005	6	123	18	11	3/8	Aug. 6 (Jul. 28–Aug. 7)	0.024 (0.000–0.053)	0.082 (0.033–0.132)	0.018 (0.00–0.070)	0.042 (0.011–0.074)	0.099 (0.047–0.150)

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^BSeroprevalence was 0.0 in fall 2003 and spring 2004, so the estimated infection rate in 2003 equaled the estimated mortality rate.

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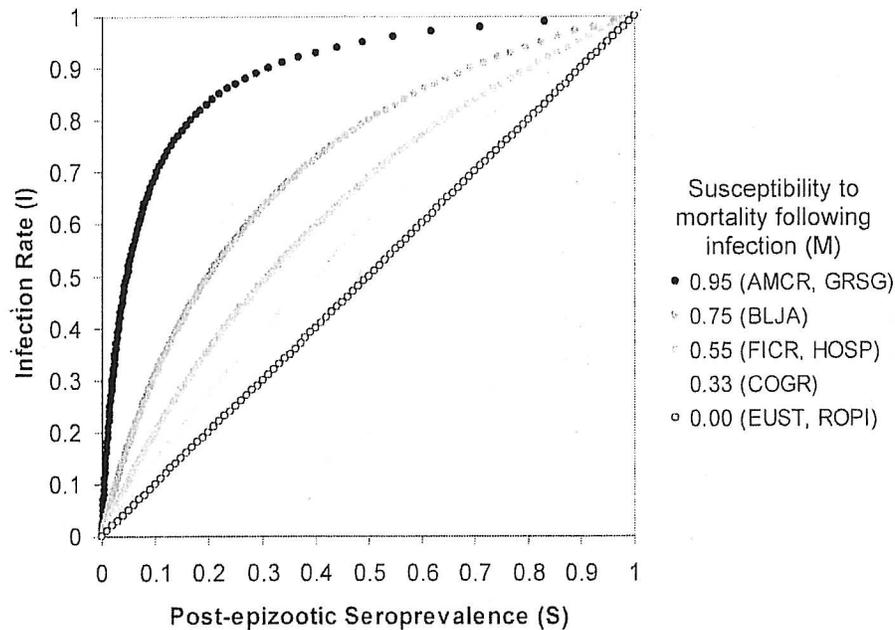
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Fig. 1. Predicted nonlinear relationships between observed postepizootic seroprevalence (S) and actual infection rate (I) over a range of susceptibility to mortality following infection (M) for representative species (4,17). Abbreviations: AMCR = American crow (*Corvus brachyrhynchos*), GRSG = greater sage-grouse (*Centrocercus urophasianus*), BLJA = blue jay (*Cyanocitta cristata*), FICR = fish crow (*Coreus ossifragus*), HOSP = house sparrow (*Passer domesticus*), COGR = common grackle (*Quiscalus quiscula*), EUST = European starling (*Sturnus vulgaris*), ROPI = rock pigeon (*Columba livia*). Divergence between postepizootic seroprevalence and infection rate increases with susceptibility and is highest at intermediate infection rates.

sizes were insufficient to provide precise estimates of mortality, seroprevalence, or infection rate on a site-by-site basis.

In this study, seroprevalence estimates underestimated infection rate by a small amount in all 3 yr of the study (Table 1). However, the relationship between infection rate and postepizootic seroprevalence was increasingly nonlinear with increasing susceptibility, and the difference between the two metrics for susceptible species was largest at intermediate infection rates (Fig. 1).

DISCUSSION

This study presents the first empirically derived estimate of actual WNV infection rate reported for any wild bird population. In this study, estimates of seroprevalence and infection rate were similar. However, this is to be expected when infection rates and seroprevalence are both low (Fig. 1). Because of the extreme susceptibility of sage-grouse, had actual infection rates been higher, the difference between observed seroprevalence and estimated infection rates would likely have been much greater. For susceptible species, seroprevalence may substantially underestimate both the prevalence and impacts of disease and confound inferences regarding exposure (e.g., habitat- or species-specific exposure rates). For example, in rock pigeons (*Columba livia*), which are largely resistant to WNV (16), seroprevalence likely provides a reliable measure of exposure (e.g., 1; Fig. 1). In contrast, for susceptible species (e.g., corvids, sage-grouse, American white pelican [*Pelecanus erythrorhynchos*], and ring-billed gull [*Larus delawarensis*]; (4,16,18)), the value of seroprevalence data for making inferences about infection rates in the absence of information on mortality is highly suspect (Fig. 1). Inferences regarding exposure in species with unknown susceptibil-

ity—including the vast majority of Nearctic and Neotropical species—may also be called into question. Despite low observed seroprevalence, sage-grouse are also considered competent amplifying hosts for WNV (4). The duration of infectious viremia in captive sage-grouse (3–5 days) was comparable to other avian species considered competent reservoirs, such as corvids, blackbirds, and raptors (16). Together, these findings underscore problems inherent with using seroprevalence as a surrogate for infection rate and for identifying competent hosts, in the absence of data on disease-related mortality.

The discovery of WNV-specific antibodies in live sage-grouse also represents the first documented evidence that individuals of this species can survive WNV infection. Seropositive birds in our study likely survived because they successfully mounted an immune response to infection. However, it is also possible that seropositive birds acquired antibodies via passive vertical transmission from their mothers (12,35). Although we observed no overt evidence of sublethal effects, if overwinter survival of infected birds was reduced compared to their uninfected counterparts, we may have underestimated infection rates.

How WNV will affect sage-grouse populations in the PRB in the future is unclear. Over the next decade, we suspect that impacts will depend less on resistance to disease than on variation in temperature (23,27,41) and changes in vector distribution (40). First, evidence suggests that resistance is neither widespread nor common. Low infection rates indicate that most sage-grouse in the PRB probably have not been exposed to WNV and therefore remain susceptible. Second, temperature strongly affects physiological and ecological processes that influence WNV transmission (7,27,41), and outbreaks are typically associated with prolonged periods of above-average

temperature and drought (10). In our study, timing and extent of mortality across years were consistent with this hypothesis, with lower mortality rates and later peaks of mortality in years with lower June–August temperatures (2004–2005) (23). Third, construction of ponds associated with coal-bed natural gas development increased larval mosquito habitat by 75% from 1999 to 2004 over a 21,000-km² area of the PRB (40). Coal-bed natural gas development is anticipated to continue in the PRB for the next 10–15 yr in occupied sage-grouse habitats.

Estimates of both seroprevalence and infection rate in our study were generally lower than seroprevalence estimates for many species in suburban, forested, farmland, urban, or wetland sites in other parts of the United States (e.g., 1,2,11,17,28,33) but similar to those in desert regions of California (27). Because of seasonal drought in summer, sagebrush-steppe may support fewer avian or mammalian hosts or fewer mosquito vectors than other, more mesic, habitats. Reservoir and amplifying hosts for WNV in this ecosystem remain unknown and likely cannot be managed over large geographic scales. For this reason, we suggest that management to reduce impacts of WNV in sage-grouse habitat focus on eliminating mosquito breeding habitat in anthropogenic water sources, particularly coal-bed natural gas ponds.

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