From the Field: Outbreak of West Nile virus in greater sage-grouse and guidelines for monitoring, handling, and submitting dead birds

Brett L. Walker, David E. Naugle, Kevin E. Doherty, and Todd E. Cornish

Abstract West Nile virus (WNV) resulted in a 25% decline in survival in four populations of radiomarked greater sage-grouse (*Centrocercus urophasianus*) across Alberta, Wyoming, and Montana in 2003. Unexpected impacts of WNV are disturbing because range-wide habitat loss and degradation already threaten sage-grouse populations. In the Powder River Basin of Wyoming and Montana, late-summer survival of sage-grouse was lower at a site with confirmed WNV mortalities (20%) than at two sites without (76%). Dramatic declines in both male and female lek attendance at the WNV site the following spring suggest that outbreaks may threaten some local populations with extirpation. The key to understanding broader impacts of WNV on sage-grouse is to monitor additional populations in 2004 and to determine whether populations infected in 2003 are again impacted this year. To facilitate this process, we describe a strategy for monitoring WNV mortality in the field and provide information on how to handle, store, and submit dead birds for testing.

Key words *Centrocercus urophasianus*, emerging infectious disease, greater sage-grouse, lek count, Montana, population decline, Powder River Basin, survival, West Nile virus, Wyoming

Since its emergence in New York state in 1999, West Nile virus (WNV) has rapidly spread west across North America and is now present in 48 of the United States (U.S.), seven Canadian provinces, Mexico, and the Caribbean (Estrada-Franco et al. 2003, Centers for Disease Control and Prevention 2004). Although population-level impacts of WNV on native species are virtually unknown (Marra et al. 2004), data from experimental infections (Komar et al. 2003) suggest that susceptibility varies by species, and that mortality rates in wild birds can be high (40–68% in American crow [*Corvus brachyrhynchos*] Caffrey et al. 2003, Naugle et al. 2004, Yaremych et al. 2004). Greater sage-grouse (*Centrocercus urophasianus*; hereafter "sage-grouse") is native to sagebrush habitats of western North America. Previously widespread, the species has been extirpated from ~50% of its original range, with an estimated range-wide population decline of 45–80% and local declines of 17–92% (Connelly and Braun 1997, Braun 1998, Connelly et al. 2000*a*, Aldridge and Brigham 2003). Loss and degradation of habitat from anthropogenic change is the most important factor leading to isolation, reduction, and extirpation of populations (Braun 1998; Connelly et al. 2000*a*,*b*; Aldridge and Brigham 2002; Knick et al.

2003), but population decline may be exacerbated by emerging infectious disease.

West Nile virus was first found in sage-grouse on 24 July 2003 in the northern Powder River Basin (PRB) of north-central Wyoming (denoted as NPRB in Naugle et al. 2004). A rapid, collaborative effort between U.S. and Canadian biologists allowed us to document impacts of WNV on survival of sage-grouse, a species endangered in Canada (Aldridge and Brigham 2003) and considered for listing under the Endangered Species Act in the U.S. Naugle et al. (2004) demonstrated that WNV reduced late-summer (Jul 1–Aug 31) survival of adult female sage-grouse by an average of 25% across four radiomarked populations in the western U.S. and Canada. Even so, survival varied among local study sites within the NPRB. Analysis of serum from 112 sage-grouse collected immediately after the outbreak failed to detect antibodies to the virus, suggesting that they lacked resistance (Naugle et al. 2004). Surveillance at three sites in the PRB indicated that the mosquito *Culex tarsalis*, a highly competent vector of WNV (Reisen and Reeves 1990, Goddard et al. 2002), was the likely culprit (Naugle et al. 2004).

Objectives of this paper are to 1) compare survival of radiomarked sage-grouse between sites with and without confirmed mortality due to WNV within the PRB, 2) evaluate whether lek counts conducted before and after the outbreak year reflect mortality rates of radiomarked birds, 3) outline a strategy for detecting WNV mortality, and 4) describe how to handle, store, and submit dead birds for testing.

Study area and methods

The PRB is an arid region of sagebrush–steppe experiencing rapid, widespread energy development. We first documented WNV in radiomarked birds during a study designed to evaluate potential impacts of coal-bed methane (CBM) development on sage-grouse. In spring 2003, we used rocket nets and spotlighting (Wakkinen et al. 1992) to capture and radiomark female sage-grouse on 3 sites. Females were fitted with necklace-type radiotransmitters with mortality switches and monitored once every 4–6 days from capture through 24 July 2003 and once every 2–3 days from 24 July–7 October 2003. Each site covered approximately 250 km^2. Study sites included rolling hills, plateaus, and valleys dominated by native sagebrush–steppe and intermixed with smaller patches of native shortgrass prairie, conifer forest, mesic shrubland, greasewood bottomlands, riparian woodland, and non-native pasture. The study site at which we detected WNV also supported extensive oil and gas development and non-irrigated agriculture. Dominant shrubs included Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) and silver sagebrush (*A. cana*). Sage-grouse at these 3 sites were non-migratory (Connelly et al. 2000*b*), showing average movements between breeding, summering, and wintering grounds of <10 km (Brett Walker, University of Montana, unpublished data).

Survival estimation

We used a Kaplan-Meier product limit estimator (Kaplan and Meier 1958) with a staggered-entry design (Pollock et al. 1989, Winterstein et al. 2001) to estimate late-summer survival of radiomarked female sage-grouse (≥1 year old). We define late summer as the period from 1 July through 31 August that coincides with peak season for WNV exposure (Naugle et al. 2004). We detected mortalities using mortality sensors on radiocollars and by visually confirming the status of each bird every 2–5 days. We used a log-rank test to evaluate differences in survival functions (Pollock et al. 1989, Winterstein et al. 2001) between a site with confirmed WNV mortalities (hereafter, "WNV site") and sites without confirmed WNV mortalities (hereafter, "non-WNV site"). Although WNV was confirmed in *C. tarsalis* captured near "non-WNV" sites, the absence of confirmed WNV mortalities and the absence of antibodies to the virus in live birds captured following the outbreak (n=48) led us to designate these as non-WNV sites.

Dead sage-grouse were tested for WNV at the Wyoming State Veterinary Laboratory (WSVL) in Laramie, Wyoming. All dead birds underwent complete necropsies and microscopic examination of routine tissues by histopathology. Each carcass was tested for WNV using two tests, real-time polymerase chain reaction (Shi 2001) and immunohistochemistry (Kiupel et al. 2003). Select cases positive for WNV were confirmed by isolation of the virus from several tissues (brain, heart, and kidney) in Vero cell cultures (Steele et al. 2000). Of 11 testable carcasses, WNV was confirmed as the cause of death in 7 cases in the PRB (Naugle et al. 2004).

Lek-count data

Changes in the number of individual sage-grouse counted at traditional lek sites are commonly used

to assess population trends (Jenni and Hartzler 1978, Emmons and Braun 1984, Connelly et al. 2000*b*). In 2003 and 2004, we conducted a total of 40 counts ($\bar{x}$=4.0 [0.7 SE] counts per lek) at 5 leks in the WNV site and 58 counts ($\bar{x}$=4.1 [0.6 SE] per lek) at 7 leks in the two non-WNV sites. We counted leks between 16 March–1 May 2003 and between 14 March–2 May 2004. These leks constitute all those known to be active in spring 2003 within the 3 study sites. We conducted counts from 0.5 hour before sunrise to 1 hour after sunrise during the spring lekking season (Jenni and Hartzler 1978, Emmons and Braun 1984). We did not conduct counts during heavy rain or when wind speed was >20 kph. We recorded date and time of survey and number of individuals by sex that attended the lek. We trained observers to identify birds to sex based on physical and behavioral characteristics.

We compared rates of male lek attendance before and after the outbreak year in the WNV and non-WNV sites by dividing number of counts in which ≥1 male was observed by total number of counts. We made similar comparisons for females by dividing number of counts in which ≥1 female was observed on a lek by total number of counts. We compared proportional change in number of males to evaluate whether lek counts conducted in the spring following the WNV outbreak reflected known mortality in radiomarked birds. We calculated proportional change for each individual lek and for all leks within a "treatment" (i.e., WNV versus non-WNV sites). We calculated proportional change for each individual lek by subtracting the absolute difference in maximum number of males counted in 2004 from the maximum count in 2003 and dividing the product by the maximum count in 2003. We substituted the sum of the maximum number of males on all leks into the same formula to calculate proportional change within a treatment. Proportional change for leks within a treatment also was calculated using the median and mean number of males to evaluate their relative influence in magnitude and direction of change.

A monitoring strategy for WNV detection

A rapid, coordinated monitoring strategy among researchers is necessary in order to develop an understanding of WNV on sage-grouse populations. We have drawn on experiences gained last year to communicate a strategy for detecting WNV mortality in field studies and for processing dead birds for testing.

Results

Monitoring

We captured and radiomarked 55 female sage-grouse in the spring of 2003. Ultimately, we tracked survival of 44 radiomarked female sage-grouse in 2003 during the late-summer period; 34 in non-WNV sites and 10 in the WNV site. Of the 10 females still alive at the WNV site on 1 July 2003, 1 was killed by a predator or scavenged, 1 struck a power line, and 6 died of WNV. Birds that died of WNV were often found intact, in good condition, and with a full crop and no external signs of trauma. We also detected outward signs of infection in the field in 3 of 6 live birds that later succumbed to WNV. Afflicted birds rarely moved far, showed tilted heads and drooping wings, and usually died within 1 to 2 days.

Survival analysis

All females that died of WNV were from 1 site; thus, we compared survival rates at the WNV site to those at the 2 non-WNV sites to assess the impact of WNV. Estimated survival of marked birds from July 1 through 31 August 2003 was higher in non-WNV sites (76%) than in the WNV site (20%) (χ^2_1= 20.7, P<0.01; Figure 1).

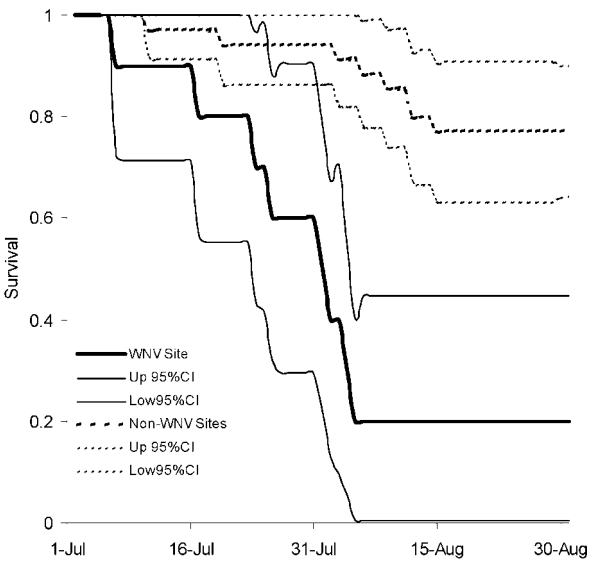

Figure 1. West Nile Virus dramatically reduced late-summer survival of female greater sage-grouse. Kaplan-Meier survival functions (±95% CIs) for radiomarked adult females at sites with (n = 10) and without (n = 34) confirmed mortality from West Nile Virus (WNV) during 1 July–31 August 2003, Powder River Basin, north-central Wyoming and southeast Montana.

Lek-count data

Number of males counted was higher at non-WNV sites ($\bar{x}$=10.2, 1.5 SE in 2003; $\bar{x}$=10.4, 1.4 SE in 2004) than at the WNV site ($\bar{x}$=5.1, 0.5 SE in 2003; $\bar{x}$=0.5, 0.2 SE in 2004) regardless of year. At least 1 male was observed in 100% of lek counts in non-WNV sites during both years and in the WNV site in 2003. At the WNV site in 2004, the number of counts in which ≥1 male was observed decreased to 33% (7/21). At least 1 female was observed in 62% (13/21) and 65% (24/37) of lek counts in non-WNV sites in 2003 and 2004, respectively. We also observed at least 1 female in 58% (11/19) of counts at the WNV site in 2003 prior to the outbreak. However, we only observed 1 female at 1 lek on a single occasion (5%; 1/21) during counts in the WNV site in 2004. The highest number of males observed at any lek at any time was 4 in the WNV site in 2004; all other individual counts of males were either 1 or 0.

Maximum number of males counted in non-WNV sites in 2004 increased on 4 leks (+16.7 to +200%), decreased on 1 lek (–28.6%), and remained constant on 2 leks (Figure 2). Maximum number of males counted in 2004 decreased on all 5 leks (–33.3 to –100%) within the WNV site (Figure 2). Proportional change from 2003–2004 increased in non-WNV sites and decreased in the WNV site, regardless of whether we used the maximum, median, or mean number of males on each lek in calculations. Proportional change in maximum, median, and mean number of males was +22.7%, +12.5%, and +10.7% in non-WNV sites and –76%, –95.4%, –91.0% in the WNV site.

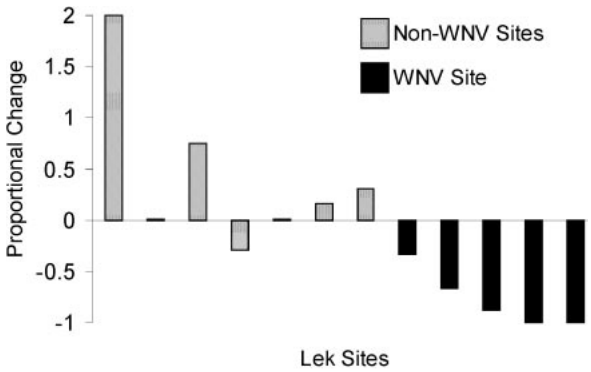

Figure 2. Male lek attendance declined the spring following the WNV outbreak. Proportional change from 2003 to 2004 in maximum number of males counted on leks at sites with and without confirmed mortality from West Nile virus (WNV) in 2003, Powder River Basin, north-central Wyoming and southeast Montana.

Discussion

Reduced survival due to the spread of WNV is disturbing because habitat loss and degradation already stress sage-grouse populations throughout the species' range. Moreover, survival of adult females has been identified as a limiting factor in population growth (Johnson and Braun 1999), and losses from WNV mortality come at a time of year when survival typically is high (Braun 1998, Schroeder et al. 1999, Connelly et al. 2000*a*, Aldridge and Brigham 2003). Survival analysis using marked sage-grouse within the PRB indicates that incidence of WNV varies widely across the landscape and can contribute to pronounced local population declines. A 95% decline in female lek attendance in the WNV site the following spring mirrored losses from WNV mortality among radiomarked individuals. A –76% decrease in maximum counts and a –95% decrease in the median count of males on leks in the WNV site suggest that males also are susceptible to high rates of mortality from WNV. Such dramatic decreases in lek counts are unlikely to have been caused by other phenomena, such as mass emigration or ongoing population decline. Movement data indicate that populations on these 3 sites are non-migratory. Indeed, 2 surviving radiomarked birds have remained on the WNV site since the outbreak. Although long-term lek-count data do indicate steady population declines across all 3 sites, the abrupt disappearance of males from multiple leks in 2004 is unprecedented (Wyoming Game and Fish Department, unpublished data; Decker Coal Company, unpublished data; Thunderbird Wildlife Consulting, Inc., unpublished data).

Long-term population-level impacts remain unclear because we do not know whether WNV will appear in other sage-grouse populations in 2004 and whether populations infected last year will again be impacted this year. We also largely lack information on susceptibility of other cohorts; 2 radiomarked chicks (*ca.* 10 weeks old) in Alberta died from WNV in 2003 (Naugle et al. 2004).

At present, the role of natural and anthropogenic sources of water in the spread of WNV is unclear, and the ecology of vectors and reservoir hosts that spread WNV in western North America remains virtually unknown. Surface water that persists into late summer in an otherwise xeric landscape may exacerbate prosperity of an exotic virus-vector complex among naïve native wildlife species. To

explore this question in detail, we initiated mosquito surveillance in the northern PRB and at 3 additional locations with radiomarked sage-grouse in Wyoming and Montana in 2004 to quantify the relative contribution of different sources of surface water to vector production.

Monitoring WNV mortality in sage-grouse

As WNV continues its westward spread in North America, a full understanding of its implications for sage-grouse populations will require a rapid, coordinated monitoring strategy among researchers. Throughout the species' range, researchers use standardized lek counts to monitor sage-grouse populations and studies of radiomarked individuals to assess various aspects of their ecology and management. Still, the intensity and timing of monitoring of radiomarked birds in most studies generally is too infrequent or occurs at the wrong time of year to assess impacts of WNV on survival. A necessary and effective strategy for detecting and assessing rates of mortality due to WNV in sage-grouse requires intensive monitoring of radiomarked birds during the peak of WNV season, typically 1 July–31 August, perhaps later in some regions. We recommend monitoring radiomarked birds at least once every 2–3 days to increase the probability that carcasses are retrieved before they decompose or are scavenged. High late-summer temperatures cause carcasses to decay rapidly, so timely recovery helps ensure that carcasses remain fresh for successful necropsy and virus testing. Researchers should also visually confirm a bird's status as dead or alive every 3–4 days, if possible. Mortality switches on radiocollars are designed to alert investigators to animals that have died, but because carcasses are often dragged or moved, a "live" radio signal does not always reliably indicate a live individual. Moreover, infected birds often can be identified in the field by a lack of mobility, a tilted or drooping head and drooping wings when roosting, or by weak flight when flushed. Radiomarked birds that succumbed to WNV in Canada moved only a few meters 2 days prior to death (Cameron Aldridge, University of Alberta, personal communication). Such birds can be followed, collected (with advance authorization from state wildlife officials), or targeted for an immediate follow-up visit. The frequency of visual confirmations must be balanced against the potential for disturbing roosting individuals or flocks. Because the peak season for WNV typically occurs after the breeding season is over (after 1 July), intensive monitoring for WNV mortality may require additional funding for salary, vehicles, housing, and telemetry flights to relocate individuals that have made large seasonal movements.

Accurately estimating mortality rates from WNV also may require additional trapping to boost sample sizes following natural attrition over the spring breeding season. Because precision in survival estimates is poor unless the number of marked birds at a given time is >20, Pollock et al. (1989) recommend that a minimum of 40–50 animals be marked at all times. Thus, researchers may wish to conduct additional trapping before the onset of WNV season.

Handling and submitting dead birds for testing

Proper handling, storage, and delivery of carcasses are important for detecting WNV, and ultimately for assessing its impacts on greater sage-grouse survival. When a dead bird is found, collect all remaining body parts, including the skeleton, viscera, head, etc. Intact carcasses are the best sample to submit for testing, and field necropsies are not recommended due to health risks to untrained personnel and potential loss or improper preservation of critical samples. Blood feathers from molting birds can be a useful sample for testing, but mature feathers are not usable. Do not submit feathers. When picking up a dead bird, invert a trash bag over your hand, pick up the bird using the bag as a barrier, and roll the bag down over the bird. Gloves may be used as an extra precaution. Label the bird with information on its identity (i.e., band number), age, sex, date, location, habitat, and collector, and place the completed label inside the bag with the carcass. Place this bag inside a second bag, and seal and label the outer bag. If birds are found freshly dead, keep carcasses refrigerated or on ice, and promptly deliver them to a testing facility using overnight or one-day shipping. Pack carcasses with ice packs to keep them cold. If carcasses must be stored for more than a day or 2, they may be frozen but should be sent in as soon as practicable.

West Nile virus sometimes can be detected in predated, scavenged, or decomposed carcasses, especially in bone marrow (found in wing and leg long bones) or any remaining visceral organs, and these carcasses or pieces should be collected and submitted for testing. Such carcasses or parts of birds should be frozen to prevent further decom-

position. To avoid delays in shipping, we recommend that researchers contact their state wildlife veterinary laboratory in advance for information on where to send carcasses for testing. One author (TEC) can provide assistance in locating a testing facility near you, and may be contacted at TCornish@uwyo.edu. Contact the testing facility by phone prior to shipment to alert them that a perishable specimen is on the way. Do not ship specimens so that they will arrive on a weekend. Most state wildlife agencies also likely will be interested in testing other game birds [e.g., sharp-tailed grouse (*Tympanuchus phasianellus*), etc.], raptors, and other avian species for WNV. Contact your state or provincial wildlife agency regarding considerations for handling, collecting, and testing other species.

As always, when conducting research in sites where WNV is known or suspected to be present, people should wear long-sleeved shirts, long pants, and other protective clothing; use mosquito repellent with DEET; and minimize exposure at dawn and dusk when feeding mosquitoes are most active. Contact your local health department for more information (e.g., Wyoming Department of Health website at http://www.badskeeter.org or the Wyoming West Nile Hotline at 1-877-WYO-BITE answered 8 am–5 pm Monday through Friday).

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Associate editor: *Ballard*