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A Serosurvey of Greater Sage-Grouse (*Centrocercus urophasianus*) in Nevada, USA

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ABSTRACT: To better understand the potential avian diseases in Greater Sage-grouse (*Centrocercus urophasianus*) in the Great Basin in Nevada, US, we collected 31 blood samples March–April 2014 and tested for antibodies to eight viruses and two bacteria. Specifically, sera were tested for antibodies to avian leukosis virus type A, B, and J (ALV-A, ALV-B, and ALV-J, respectively), infectious bursal disease virus, infectious bronchitis virus, reticuloendothelial virus, avian influenza virus (AIV), West Nile virus, *Pasteurella multocida* (PM), and *Salmonella enterica* serovar Pullorum. Serum antibodies against ALV-A and -B (1/31, 3%), ALV-J (5/31, 16%), PM (1/31, 3%), and AIV (2/31, 6%) were detected by enzyme-linked immunosorbent assay (ELISA). While ELISA tests used have only been validated in domestic poultry, the serologic data should be used as a potential indicator of the range of bacterial and viral infectious agents that can infect the Greater Sage-grouse.

Key words: Avian influenza, *Centrocercus urophasianus*, Greater Sage-grouse, infectious disease.

The Greater Sage-grouse (*Centrocercus urophasianus*) is the largest grouse species in North America. Although information is limited, there is a general consensus that the population as a whole has steadily declined (Garton et al. 2011). Studies that focus on identifying threats to Greater Sage-grouse (henceforth referred to as GRSG) can help guide management and conservation actions within specific ecoregions. Major threats to GRSG populations include prescribed fire (Nelle et al. 2000), wildfire (Coates et al. 2015), invasive grass (Lockyer et al. 2015), anthropogenic development (Walker et al. 2007a), conifer expansion (Baruch-Mordo et al. 2013), and predation (Hagen 2011). Less is known about avian diseases that may adversely affect GRSG populations (Christiansen and Tate 2011). While antibody is only indicative of seroconversion as opposed to disease, presence of antibody demonstrates previous exposure. Therefore, antibody prevalence studies are a crucial first step in assessing the exposure of animals to infectious agents. We identified exposure to eight previously documented (i.e., clinical disease or presence of antibody) infectious viruses and bacteria known to be present in the Tetraonidae subfamily or greater Phasianidae family (Drew et al. 1998; Peterson et al. 1998, 2002; Dimcheff et al. 2000; Walker et al. 2007b).

In March and April 2014, blood samples were collected from 31 sage-grouse captured at six breeding sites (leks) representing a large geographic portion of the Great Basin. These samples were collected and geocoded (Fig. 1) in conjunction with a large-scale study evaluating spatiotemporal variation in GRSG demographics. The six sites (from west to east) were identified as Virginia Mountains (VM), Desatoya Mountains (DM), White Pine Range (WP), Tuscarora Mountains (TM), McGinness Hills (MH), and Egan Range (EG; Fig. 1). Birds were captured using spotlighting techniques (Wakkinen et al. 1992), and blood and sera were collected as described by Owen (2011). Sex and age class were determined using plumage characteristics (Eng 1955).

Eight viruses and two bacteria (Table 1) were selected for serologic testing based on their previous documented presence in GRSG or other wild avian species in the Tetraonidae or Phasianidae. Serum samples were analyzed by an enzyme-linked immunosorbent assay (ELISA; IDEXX Laboratories, Inc., Westbrook, Maine, USA) for antibodies to avian influenza virus (AIV), avian leukosis virus

FIGURE 1. Greater Sage-grouse (*Centrocercus urophasianus*) habitat in Nevada, USA sampling areas and locations of antibody-positive grouse. Gray shading represents habitat of sage-grouse (Coates et al. 2015). Open circles indicate study site trapping locations from west to east: Virginia Mountains, Desatoya Mountains, McGinness Hills, Tuscarora Mountains, Egan Range, and White Pine Range. Dots show sites where grouse had detectable antibody: A=avian leukosis virus subtype J, B=avian leukosis virus subtypes A and B, C=*Pasteurella multocida*, D=avian influenza virus.

subtypes A and B (ALV-A, ALV-B), avian leukosis virus subtype J (ALV-J), *Pasteurella multocida* (PM), infectious bursal disease virus (IBDV), reticuloendothelial virus (REV), and infectious bronchitis virus (IBV). Samples with positive AIV serum titers were confirmed positive via agar gel immune diffusion and by neuraminidase-inhibition and hemagglutination-inhibition assays for further subtyping and confirmatory analyses (National Veterinary Services Laboratory [NVSL], Ames, Iowa, USA). The NVSL also tested the AI antibody-positive samples by a plaque reduction neutralization test for WNV. The remaining

TABLE 1. Results of tests for antibodies against various pathogens in 31 Greater Sage-grouse (*Centrocercus urophasianus*) captured March–April 2014 at six lekking sites in Nevada, USA.[a]

Pathogen	No. positive/ total (%)	No. positive/*n* (%)								Age class of positives
		VM ($n = 1$)	DM ($n = 1$)	WP ($n = 7$)	TM ($n = 9$)	MH ($n = 7$)	EG ($n = 6$)	Males ($n = 30$)	Females ($n = 1$)	
Avian influenza virus	2/31 (7)	0	0	2/6 (33)	0	0	0	2/30 (7)	0	Adult (both)
Avian leukosis virus subtypes A and B	1/31 (3)	0	0	0	0	1/7 (14)	0	1/30 (3)	0	Adult
Avian leukosis virus subtype J	5/31 (16)	1/1 (100)	0	0	2/9 (22)	2/7 (29)	0	5/30 (17)	0	Adult (3) and yearling (2)
Infectious bronchitis virus	0	0	0	0	0	0	0	0	0	NA
Infectious bursal disease virus	0	0	0	0	0	0	0	0	0	NA
Pastuerella multocida	1/31 (3)	0	0	0	1/9 (11)	0	0	1/30 (3)	0	Yearling (1)
Reticuloendothelial virus	0	0	0	0	0	0	0	0	0	NA
West Nile virus	0	0	0	0	0	0	0	0	0	NA
Samonella Pullorum	0	0	0	0	0	0	0	0	0	NA

[a] VM = Virginia Mountains; DM = Desatoya Mountains; WP = White Pine Range; TM = Tuscarora Mountains; MH = McGinness Hills; EG = Egan Range; NA = not applicable.

samples were tested for WNV antibodies at the Center for Vector-Borne Diseases (Davis, California, USA) using an in-house developed ELISA specific for avian serum (Ebel et al. 2002). Samples were tested for antibodies against *Salmonella enterica* serovar Pullorum (SP) using a microagglutination test.

Locations of positive samples were mapped using ArcGIS version 12.2 (ESRI, Redlands, California, USA) (Fig. 1). Descriptive statistics of the sample population were calculated based on age class, sex, and location of capture to characterize the prevalence of potential pathogen exposure (Table 1).

All but one of the 31 GRSG sampled were male. There were 22 adults (71%), seven yearlings (23%), and two birds of unknown age (7%). We captured 3% of our population at VM, 3% from DM, 23% from WP, 29% from TM, 23% from MH, and 19% from EG (Table 1). Serum samples had detectable antibody to AIV, ALV-A, ALV-B, ALV-J, and PM (Table 1). All samples were negative for IBDV, IBV, REV, WNV, and SP. Numbers, sexes, and age classes of positive GRSG for each pathogen are provided in Table 1. One of the two positive AIV samples was positive for the H7 hemagglutinin subtype. The neuraminidase subtype was tested by NVSL but the results were inconclusive. Three adult males and two yearling males were positive for ALV-J antibodies at MH, TM, and the VM (Table 1 and Fig. 1). One of the MH samples found positive to ALV-J was also positive for ALV-A and ALV-B (Fig. 1). One yearling male at TM was positive for antibodies associated with one of the 16 serotypes of PM (Table 1 and Fig. 1).

To our knowledge, these findings represent the first evidence of any sage-grouse exposure to AIV. While our results do not indicate whether the H7 was a high or low pathogenic AI, it brings into question how GRSG could have been exposed to H7 AIV in the Great Basin. The primary reservoirs of AIV are waterfowl. The Great Basin is on the Pacific Flyway and it has been identified as a targeted watershed with respect to movement of dabbling ducks and influenza A (US Department of Agriculture 2015); therefore, the potential for exposure between waterfowl—potential carriers of AIV—and nonwaterfowl exists in this region. For example, a highly pathogenic strain of H5N8 was found in a Mallard (*Anas platyrhynchos*) in southeastern Nevada in January 2015 (Promed 2015).

Five birds were positive for ALV-J antibody. In domestic poultry, ALV-J is a more virulent form of ALV. Our literature search identified one clinical case of endogenous ALV infection in the GRSG in Colorado (Dimcheff et al. 2000). To our knowledge, this is the first report of antibody to ALV-J in either sage-grouse species. While reports of ALV-A, ALV-B, and ALV-J in any wild avian species is rare, ALV-A and -B were isolated from 10 wild avian species in China (Li et al. 2013) and cause neoplastic and reproduction problems in poultry worldwide.

Pasteurella multocida, the causative agent of avian cholera, has a worldwide distribution (including most of the US) and produces septicemic and respiratory disease in >180 species of wild birds. Exposure could come from a variety of sources including wetlands, cattle, corvids, and rodents (Coates et al. 2008). Serotyping is necessary to further identify the significance of PM in the GRSG because only serotypes 1 and 3 are commonly pathogenic.

While there are reports of exposure to WNV (Walker et al. 2007b) in Tetraonidae, we did not detect WNV antibody. This could be due to small sample size, lack of exposure, or the test's lack of reactivity with sage-grouse serum. Because WNV is known to cause up to 28.9% mortality in GRSG, it is also possible that GRSG exposed to WNV were dead and hence not tested (Walker et al. 2007b).

Although we detected antibody to AIV and ALV-J in GRSG, due to the small sample size, potential for false ELISA positives, limited time of the study, and limited geographic range, further surveillance is warranted. Land-based birds are potential intermediaries in the emergence of new strains of influenza A viruses (Delogu et al. 2013); therefore, surveillance is essential to determine the role GRSG might play in the complex ecology of AI in the Great Basin. Furthermore, estimating the spatiotemporal patterns of disease via a longitudinal study would provide further

insights regarding disease incidence rates and its influence on population vital rates (e.g., survival), which would help characterize the role that select infectious agents have in GRSG populations.

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