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## Infectious Disease Survey of Lesser Prairie Chickens in North Texas

Markus J. Peterson,<sup>1,4</sup> Pamela J. Ferro,<sup>2</sup> M. Nils Peterson,<sup>1</sup> Robert M. Sullivan,<sup>3</sup> Benjamin E. Toole,<sup>1</sup> and Nova J. Silvy<sup>1</sup> <sup>1</sup> Department of Wildlife and Fisheries Sciences, Texas A&M University, TAMU-2258, College Station, Texas 77843-2258, USA; <sup>2</sup> Texas Veterinary Medical Diagnostic Laboratory System, P.O. Drawer 3040, College Station, Texas 77841-6040, USA; <sup>3</sup> Texas Parks and Wildlife Department, P.O. Box 659, 5900 Cemetery Road, Canyon, Texas 79015, USA; <sup>4</sup> Corresponding author (email: mpeterson@tamu.edu)

**ABSTRACT:** Lesser prairie chicken (*Tympanuchus pallidicinctus*) abundance, like that of most grassland birds, has declined rangewide for decades. Although habitat loss and degradation are likely ultimate causes for this decline, infectious agents, particularly microparasites, could be proximate contributors. No surveys of pathogenic bacteria or viruses have been published for this species. We surveyed 24 free-living lesser prairie chickens from Hemphill County, Texas (USA), for evidence of exposure to *Salmonella typhimurium*, *S. pullorum*, *Mycoplasma gallisepticum*, *M. synoviae*, *Chlamydophila psittaci*, and the avian influenza, Newcastle disease, infectious bronchitis, and reticuloendotheliosis viruses. Two of 18, and eight of 17 samples were seropositive for the Massachusetts and Arkansas serotypes of infectious bronchitis virus, respectively. Five of the eight positive individuals were juveniles, two of which were seropositive for both serotypes. All other serologic and genetic tests were negative. Because the ecological significance of these results is unknown, the pathogenesis, transmission, and/or population-level influences of infectious bronchitis and related avian coronaviruses for lesser prairie chickens deserves further study.

**Key words:** Avian coronavirus, infectious bronchitis virus, infectious disease, lesser prairie chicken, serologic survey, Texas, *Tympanuchus pallidicinctus*.

Recently, conservation scientists have voiced concern regarding apparent broad-scale declines in abundance of most grassland bird species (e.g., Knopf, 1994; Peterjohn and Sauer, 1999). The lesser prairie chicken (*Tympanuchus pallidicinctus*) exemplifies this situation. Lesser prairie chicken abundance declined dramatically from about 1880 to 1980 (Crawford, 1980; Taylor and Guthery, 1980), and although it increased somewhat during the mid-1980s, the 1990s were again characterized by declining abundance (Giesen, 1998). For this reason, the US Fish and Wildlife Service ruled in 1998 that listing the lesser prairie

chicken as threatened under the US Endangered Species Act of 1973 was warranted but precluded by higher listing priorities (50 CFR 17). Comprehensive reviews of previous research support the contention that habitat loss and conversion were ultimately responsible for this broad-scale decline in abundance (Crawford, 1980; Taylor and Guthery, 1980; Giesen, 1998). Wildlife managers, however, have suggested that infectious agents might be one of several proximate contributors to these declines (Mote et al., 1999).

Emerson (1951) reported the presence of the Mallophaga, *Goniodes cupido*, and *Lagopoecus* sp. for an unrecorded number of lesser prairie chickens collected in Oklahoma (USA). Addison and Anderson (1969), also in Oklahoma, examined four lesser prairie chickens and identified the eye worm, *Oxyspirura petrowi*, under the nictitating membranes of at least one of these birds. Similarly, Pence and Sell (1979) found *O. petrowi* in four of seven lesser prairie chickens from the western panhandle of Texas (USA). They also identified *Heterakis isolonche* and *Rhabdometra odiosa* from six and three of 10 samples, respectively, from the same location. Stabler (1978) found *Plasmodium* sp. on blood films from two of 29 and two of eight lesser prairie chickens from New Mexico (USA) and Texas, respectively. No surveys designed to detect lesser prairie chicken exposure to other microparasitic agents have been published. Free-living Atwater's prairie chickens (*T. cupido atwateri*), a closely related species endemic to coastal Texas prairies characterized by comparatively high precipitation (Peterson, 1996), were positive for specific antibody to *Pasteurella multocida* (Peterson et

al., 1998). All positive individuals were captured in areas where outbreaks of avian cholera in waterfowl had recently occurred. Specific antibody to reticuloendotheliosis virus also was found in free-living Attwater's prairie chickens, and the virus isolated from clinically ill Attwater's and greater prairie chickens (*T. cupido pinna-tus*) held in captivity (Drew et al., 1998).

Because microparasites potentially could limit lesser prairie chicken populations, and the only existing microparasitic survey dealt with hemoparasites (Stabler, 1978), we surveyed free-living lesser prairie chickens from Hemphill County, Texas, for evidence of exposure to 10 additional microparasitic agents known to cause disease in galliforms. There is no commercial poultry industry in this area, although some ranch managers maintain poultry for their own use.

Lesser prairie chickens were captured during the spring (April–May) on leks in Hemphill County, Texas (35°58'N, 100°08'W; 36°00'N, 100°14'W), part of the northern Rolling Plains physiographic region (Gould, 1962), using rocket nets (Schemnitz, 1994) and drop nets (Silvy et al., 1990) in 1997 and 2001, respectively. We banded each captured prairie chicken with a numbered, blue anodized aluminum leg band, attached a radio transmitter, recorded each individual's sex, body mass, and age as either juvenile or adult using Ammann's (1944) outer primary technique. A 2 ml blood sample was taken via jugular venipuncture from each prairie chicken. Approximately 0.25 ml of each sample was immediately placed in a heparinized tube (Capiject; Terumo Medical Corporation, Elkton, Maryland, USA) and the remainder in a heparinized evacuated tube. Each afternoon, the vacuum tubes were centrifuged and plasma placed in sterile vials ( $\leq 3$  hr). Both the plasma and whole blood samples were held at approximately  $-20$  C pending analysis ( $\leq 10$  days).

Samples were submitted to the Texas Veterinary Medical Diagnostic Laboratory

(TVMDL; College Station, Texas) for serologic and genetic testing. Serologic testing was conducted for specific antibody to *Salmonella typhimurium* and *S. pullorum* using tube agglutination tests (Veterinary Services, 2000), with antigens obtained from the University of Minnesota (St. Paul, Minnesota, USA). Serum plate agglutination tests (Veterinary Services, 2000), with antigens from Intervet America (Millsboro, Delaware, USA), were performed for both *Mycoplasma gallisepticum* and *M. synoviae* testing. Samples were tested for *Chlamydophila psittici*-specific antibody (IgM) using an elementary body agglutination test (Grimes et al., 1994). Antigen was prepared by TVMDL following Grimes et al. (1994), and titers  $\geq 1:20$  were considered positive. An agar gel immunodiffusion assay (Veterinary Services, 2000), using antigen and specific antisera obtained from the National Veterinary Services Laboratory (Ames, Iowa, USA), was used for screening plasma samples for avian influenza virus specific antibody. Microhemagglutination-inhibition tests were used for detecting antibodies to Newcastle disease (Beard and Wilkes, 1973) and infectious bronchitis virus (Massachusetts and Arkansas serotypes; Alexander and Chettle, 1977), with titers  $\geq 1:10$  considered positive. Antigens were obtained from the National Veterinary Services Laboratory for Newcastle disease and from SPAFAS, Inc. (Preston, Connecticut, USA) for infectious bronchitis. Whole blood samples were screened for reticuloendotheliosis virus proviral DNA using a polymerase chain reaction (PCR; Aly et al., 1993; Davidson et al., 1995).

Blood samples were obtained from 24 apparently healthy lesser prairie chickens (seven in 1997; 17 in 2001). Because trapping on leks favored capturing adult males, and evidence of exposure to certain infectious agents could differ with age, we provided the number of birds tested by sex and age for reader convenience (Table 1). Because all samples were PCR negative for reticuloendotheliosis virus proviral

TABLE 1. Number of lesser prairie chickens from Hemphill County, Texas, positive ( $n$  tested) for 1) specific antibody to *Salmonella typhimurium* (*S. typ.*), *S. pullorum* (*S. pul.*), *Mycoplasma gallisepticum* (*M. gal.*), *M. synoviae* (*M. syn.*), *Chlamydophila psittici* (*Chlam.*), and avian influenza (AIV), Newcastle disease (NDV), infectious bronchitis (IBV; both Massachusetts [Mass.] and Arkansas [Ark.] serotypes) viruses; and 2) reticuloendotheliosis virus (REV) proviral DNA by PCR.

Sex <sup>a</sup>	Age <sup>b</sup>	Serology							IBV		
		<i>S. typ.</i>	<i>S. pul.</i>	<i>M. gal.</i>	<i>M. syn.</i>	<i>Chlam.</i>	AIV	NDV	Mass.	Ark.	REV
F	J	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)	1 (4)	3 (4)	0 (4)
F	A	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)			0 (1)
M	J	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	1 (2)	2 (2)	0 (3)
M	A	0 (16)	0 (16)	0 (16)	0 (16)	0 (16)	0 (16)	0 (16)	0 (12)	3 (11)	0 (16)
Total		0 (24)	0 (24)	0 (24)	0 (24)	0 (24)	0 (24)	0 (23)	2 (18)	8 (17)	0 (24)

<sup>a</sup> F = female, M = male.

<sup>b</sup> J = Juvenile, A = adult.

DNA, virus isolation was not attempted. Two individuals were seropositive for the Massachusetts, and eight for the Arkansas serotype of avian infectious bronchitis virus (Table 1; range of titers 1:10–1:40). All positive birds were sampled during 2001, and five of eight positive individuals were juveniles. A juvenile male and female were positive for antibodies to both serotypes. All other serologic tests were negative (Table 1).

In domestic chickens, avian infectious bronchitis virus, a coronavirus, causes acute, highly contagious upper respiratory disease, decreased egg production and quality, decreased growth rates, and mortality as high as 25% in chicks <6 wk of age (Cavanagh and Naqi, 1997). Domestic chickens are considered to be the only host naturally infected by infectious bronchitis virus and where this virus caused disease (Cavanagh and Naqi, 1997; Cavanagh, 2001). Coronaviruses, however, also have been isolated from domestic turkeys (Nagaraja and Pomeroy, 1997) and pheasants (Spackman and Cameron, 1983; Gough et al., 1996).

Traditionally, turkey coronavirus, pheasant coronavirus, and infectious bronchitis were considered distinct, if not separate species (Cavanagh, 2001). Recent genetic characterization, however, found no unique features of turkey or pheasant isolates that permit unequivocal differentia-

tion from infectious bronchitis viruses (Guy, 2000; Cavanagh, 2001; Cavanagh et al., 2001). It now is unclear whether avian coronaviruses isolated from turkeys, pheasants, and chickens should be considered separate coronavirus species, or whether all avian coronaviruses having similar gene sequences are simply host-range variants of a single species (Cavanagh, 2001). Further, both California quail (*Callipepla californica*) and domestic European and Japanese quail were susceptible to challenge with infectious bronchitis virus (Edgar and Waggoner, 1964; Biondi and Schirvo, 1966; Allred et al., 1973). Conversely, more distantly related small passerine birds, including the Eurasian blackbird (*Turdus merula*), European starling (*Sturnus vulgaris*), common chaffinch (*Fringilla coelebs*), European goldfinch (*Carduelis carduelis*), European greenfinch (*C. chloris*), Eurasian linnet (*C. canabina*), common canary (*Serinus canaria*), and house sparrow (*Passer domesticus*) were not susceptible to challenge with infectious bronchitis virus (Biondi and Schirvo, 1966; Allred et al., 1973). Thus it seems probable that host specificity of avian coronaviruses is, at least in part, a function of taxonomic distance between host species.

Because the hemagglutination-inhibition test used for our infectious bronchitis screening is unlikely to produce false pos-

itive reactions (Alexander and Chettle, 1977), it appears that lesser prairie chickens in Hemphill County, Texas, have been exposed to an avian coronavirus antigenically similar to the Arkansas serotype of infectious bronchitis virus. Theoretically, infectious agents having mild to moderate pathogenicity, particularly those that decrease reproductive productivity are most likely to limit host abundance in wild populations (Anderson and May, 1979, 1981; Bowers et al., 1993). Because lesser prairie chicken hens hatch no more than one clutch annually (Giesen, 1998), if an avian coronavirus infection were to depress egg production, reduce weight gain in chicks, or cause significant chick mortality—as infectious bronchitis does in domestic chickens—it could contribute to declining prairie chicken abundance. Considering the tenuous future of lesser prairie chicken populations, this potentiality should be pursued.

Lesser prairie chickens belong to the avian family Phasianidae, as do chickens, turkeys, pheasants, and Old World quails. Thus, they could be susceptible to infectious bronchitis virus, other known avian coronaviruses, or have their own coronavirus (Cavanagh, 2001). A logical first step in understanding the implications of exposure to the virus would be to challenge captive-reared lesser prairie chickens with an Arkansas isolate of infectious bronchitis virus, then describe pathogenesis and virus transmission should this species prove susceptible. Further, if clinically ill birds could be obtained from the wild, virus isolation and characterization should be attempted. Answers to these questions could be critical to current conservation efforts as well as anticipated captive rearing and reintroduction programs in west Texas.

Because existing surveys for infectious agents of lesser prairie chickens are characterized by limited sample sizes and geographic extents (e.g., Addison and Anderson, 1969; Stabler, 1978; Pence and Sell, 1979; this study), there is a need for systematic surveys conducted across this spe-

cies' range that could accurately estimate the prevalence of these agents. This is particularly true for diseases that might be population limiting, such as certain hemoparasites, intestinal protozoans, and other microparasitic agents known to be pathogenic to gallinaceous birds. We suggest that screening for avian infectious bronchitis virus/avian coronavirus should be part of such efforts.

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