Journal of Wildlife Diseases, 38(4), 2002, pp. 834–839

Infectious Disease Survey of Lesser Prairie Chickens in North Texas

Markus J. Peterson,[1,4] **Pamela J. Ferro,**[2] **M. Nils Peterson,**[1] **Robert M. Sullivan,**[3] **Benjamin E. Toole,**[1] **and Nova J. Silvy**[1] [1] Department of Wildlife and Fisheries Sciences, Texas A&M University, TAMU-2258, College Station, Texas 77843-2258, USA; [2] Texas Veterinary Medical Diagnostic Laboratory System, P.O. Drawer 3040, College Station, Texas 77841-6040, USA; [3] Texas Parks and Wildlife Department, P.O. Box 659, 5900 Cemetery Road, Canyon, Texas 79015, USA; [4] 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 Attwater's prairie chickens (*T. cupido attwateri*), 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 pinnatus*) 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 (≤3 hr). Both the plasma and whole blood samples were held at approximately −20 C pending analysis (≤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 ≥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 ≥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.

		Serology									
									IBV		
Sex[a]	Age[b]	*S. typ.*	*S. pul.*	*M. gal.*	*M. syn.*	*Chlam.*	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)
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)

[a] F = female, M = male.
[b] 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 differentiation 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. cannabina*), 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 species' 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.

We thank D. S. Davis, T. W. Hinkle, J. P. Hughes, K. D. Mote, and D. A. Swepston for their advice and/or help with the trapping. We appreciate the assistance of T. L. Lester, J. A. Linares, L. W. Sneed, and W. L. Wigle with the serologic and genetic testing. This paper benefited from critical evaluation by two anonymous reviewers. Texas Parks and Wildlife Department, Federal Aid in Wildlife Restoration Project W-126-R, the Institute for Science, Technology, and Public Policy in the George Bush School of Government and Public Service, the Texas Agricultural Experiment Station, The Schubot Exotic Bird Health Center, and Texas A&M University supported this project.

LITERATURE CITED

ADDISON, E. M., AND R. C. ANDERSON. 1969. *Oxyspirura lumsdeni* n. sp. (Nematoda: Thelaziidae) from Tetraonidae in North America. Canadian Journal of Zoology 47: 1223–1227.

ALEXANDER, D. J., AND N. J. CHETTLE. 1977. Procedures for the haemagglutination and the haemagglutination-inhibition tests for avian infectious bronchitis virus. Avian Pathology 6: 9–17.

ALLRED, J. N., L. G. RAGGI, AND G. G. LEE. 1973. Susceptibility and resistance of pheasants, starlings, and quail to three respiratory diseases of chickens. California Fish and Game 59: 161–167.

ALY, M. M., E. J. SMITH, AND A. M. FADLY. 1993. Detection of reticuloendotheliosis virus infection using the polymerase chain reaction. Avian Pathology 22: 543–554.

AMMANN, G. A. 1944. Determining the age of pinnated and sharp-tailed grouse. The Journal of Wildlife Management 8: 170–171.

ANDERSON, R. M., AND R. M. MAY. 1979. Population biology of infectious diseases: Part I. Nature 280: 361–367.

———, AND ———. 1981. The population dynamics of microparasites and their invertebrate hosts.

Philosophical Transactions of the Royal Society of London B 291: 451–524.

BEARD, C. W., AND W. J. WILKES. 1973. A simple and rapid microtest procedure for determining Newcastle hemagglutination-inhibition (HI) antibody titers. Proceedings of the annual meeting, US Animal Health Association 77: 596–600.

BIONDI, E. AND A. SCHIRVO. 1966. Investigations into the susceptibility of various small passerine birds to the virus of infectious bronchitis. Acta Medica Veterinaria 12: 537–547.

BOWERS, R. G., M. BEGON, AND D. E. HODGKINSON. 1993. Host-pathogen population cycles in forest insects?: Lessons from simple models reconsidered. Oikos 67: 529–538.

CAVANAGH, D. 2001. A nomenclature for avian coronavirus isolates and the question of species status. Avian Pathology 30: 109–115.

———, AND S. A. NAQI. 1997. Infectious bronchitis. *In* Diseases of poultry. 10th Edition, B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald and Y. M. Saif (eds.). Iowa State University Press, Ames, Iowa, pp. 511–526.

———, K. MAWDITT, M. SHARMA, S. E. DRURY, H. L. AINSWORTH, P. BRITTON, AND R. E. GOUGH. 2001. Detection of a coronavirus from turkey poults in Europe genetically related to infectious bronchitis virus of chickens. Avian Pathology 30: 355–368.

CRAWFORD, J. A. 1980. Status, problems, and research needs of the lesser prairie chicken. *In* Proceedings of the Prairie Grouse Symposium, P. A. Vohs, Jr. and F. L. Knopf (eds.). Oklahoma State University, Stillwater, Oklahoma, pp. 1–7.

DAVIDSON, I., A. BOROVSDAYA, S. PERL, AND M. MALKINSON. 1995. Use of the polymerase chain reaction for the diagnosis of natural infection of chickens and turkeys with Marek's disease virus and reticuloendotheliosis virus. Avian Pathology 24: 69–94.

DREW, M. L., W. L. WIGLE, D. L. GRAHAM, C. P. GRIFFIN, N. J. SILVY, A. M. FADLY, AND R. L. WITTER. 1998. Reticuloendotheliosis in captive greater and Attwater's prairie chickens. Journal of Wildlife Diseases 34: 783–791.

EDGAR, S. A., AND R. WAGGONER. 1964. Pathogens of *Coturnix coturnix japonica.* Quail Quarterly 2: 11–14.

EMERSON, K. C. 1951. A list of Mallophaga from gallinaceous birds of North America. The Journal of Wildlife Management 15: 193–195.

GIESEN, K. M. 1998. Lesser prairie chicken. *In* The birds of North America, No. 364, A. Poole and F. Gill (eds.). The Birds of North America, Philadelphia, Pennsylvania, 20 pp.

GOUGH, R. E., W. J. COX, C. E. WINKLER, M. W. SHARP, AND D. SPACKMAN. 1996. Isolation and identification of infectious bronchitis virus from pheasants. Veterinary Record 138: 208–209.

GOULD, F. W. 1962. Texas plants—A checklist and ecological summary. Publication MP-585, Texas Agricultural Experiment Station, The Agricultural and Mechanical College of Texas, College Station, 112 pp.

GRIMES, J. E., T. N. TULLY, JR., F. ARIZMENDI, AND D. N. PHALEN. 1994. Elementary body agglutination for rapidly demonstrating chlamydial agglutinins in avian serum with emphasis on testing cockatiels. Avian Diseases 38: 822–831.

GUY, J. S. 2000. Turkey coronavirus is more closely related to avian infectious bronchitis virus than to mammalian coronaviruses: A review. Avian Pathology 29: 207–212.

KNOPF, F. L. 1994. Avian assemblages on altered grasslands. Studies in Avian Biology 15: 247–257.

MOTE, K. D., R. D. APPLEGATE, J. A. BAILEY, K. E. GIESEN, R. HORTON, AND J. L. SHEPPARD. 1999. Assessment and conservation strategy for the lesser prairie-chicken (*Tympanuchus pallidicinctus*). Kansas Department of Wildlife and Parks, Emporia, Kansas, pp. 51.

NAGARAJA, K. V., AND B. S. POMEROY. 1997. Coronaviral enteritis of turkeys (bluecomb disease). *In* Diseases of poultry, 10th Edition, B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald and Y. M. Saif (eds.). Iowa State University Press, Ames, Iowa, pp. 686–692.

PENCE, D. B., AND D. L. SELL. 1979. Helminths of the lesser prairie chicken, *Tympanuchus pallidicinctus* (Ridgway) (Tetraonidae), from the Texas panhandle. Proceedings of the Helminthological Society of Washington 46: 146–149.

PETERJOHN, B. G., AND J. R. SAUER. 1999. Population status of North American grassland birds from the North American Breeding Bird Survey 1966–1996. Studies in Avian Biology 19: 27–44.

PETERSON, M. J. 1996. The endangered Attwater's prairie chicken and an analysis of prairie grouse helminthic endoparasitism. Ecography 19: 424–431.

———, J. R. PURVIS, J. R. LICHTENFELS, T. M. CRAIG, N. O. DRONEN, JR., AND N. J. SILVY. 1998. Serologic and parasitologic survey of the endangered Attwater's prairie chicken. Journal of Wildlife Diseases 34: 137–144.

SCHEMNITZ, S. D. 1994. Capturing and handling wild animals. *In* Research and management techniques for wildlife and habits, T. A. Bookhout (ed.). The Wildlife Society, Bethesda, Maryland, pp. 106–124.

SILVY, N. J., M. E. MORROW, E. SHANELY, JR., AND R. D. SLACK. 1990. An improved drop net for capturing wildlife. Proceedings of the annual conference, Southeastern Association of Fish and Wildlife Agencies 44: 374–378.

SPACKMAN, D., AND I. R. D. CAMERON. 1983. Isolation of infectious bronchitis virus from pheasants. The Veterinary Record 113: 354–355.

STABLER, R. M. 1978. *Plasmodium* (*Giovannolaia*) *pedioecetii* from the lesser prairie chicken, *Tym-*

panuchus pallidicinctus. Journal of Parasitology 64: 1135–1126.

TAYLOR, M. A., AND F. S. GUTHERY. 1980. Status, ecology, and management of the lesser prairie chicken. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-77, Fort Collins, Colorado, 15 pp.

VETERINARY SERVICES. 2000. National poultry improvement plan and auxiliary provisions. Veterinary Services, Animal and Plant Inspection Service, U.S. Department of Agriculture, Conyers, Georgia, 111 pp.

Received for publication 30 October 2001.