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Survey for Reticuloendotheliosis Viruses in Wild Populations of Greater and Lesser Prairie-Chickens

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ABSTRACT.—Reticuloendotheliosis (RE) is a viral disease documented from poultry, which has been found to cause mortality in captive Attwater's (*Tympanuchus cupido attwateri*) and Greater (*T. c. pinnatus*) prairie-chickens. We surveyed blood samples from 354 Greater Prairie-Chickens from seven states collected during 1998, 1999, and 2000, and from 184 Lesser Prairie-Chickens (*T. pallidicinctus*) from three states during 1999 and 2000, for the presence of RE virus proviral DNA using a polymerase chain reaction (PCR) test. All samples were negative for the presence of RE virus proviral DNA except for two samples collected from male Greater Prairie-Chickens taken in Oklahoma during 1998. This suggests that RE may not be a serious problem for most wild populations of prairie-chickens. Although our results were largely negative, because of the serious consequences of RE, the presence of the disease in wild populations of prairie-chickens should be carefully considered in any future relocation and reintroduction efforts. *Received 30 July 2001, accepted 26 April 2002.*

Reticuloendotheliosis (RE) is a disease of a number of avian species, including domestic chickens (*Gallus gallus*), ducks, quail, pheasants, and domestic turkeys (*Meleagris gallopavo*; Bagust 1993, Witter 1997). RE has been found to cause morbidity and mortality in captive Greater and Attwater's prairie-chickens (*Tympanuchus cupido pinnatus* and *T. c. attwateri*; Drew et al. 1998).

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Populations of the two species of prairie-chicken, Greater Prairie-Chicken (including the Attwater's subspecies) and Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*) have declined dramatically during recent years (Westemeier and Gough 1999). It is not clear in many cases why the declines have occurred. Because of the serious effects of RE on captive prairie-chickens (Drew et al. 1998), it is important to determine if RE is a potential cause for the declines of wild populations. As pointed out by Friend et al. (2001), the ability to evaluate the effects of disease on a free-ranging bird species is fraught with difficulties. However, to begin to address the issue of whether RE was present in the wild populations, we surveyed for the presence of the disease in prairie-chickens across their range using samples collected during 1998, 1999, and 2000.

METHODS

We followed generally accepted procedures (Gaunt et al. 1999) for handling animals and obtaining samples. We collected about 1 ml of blood from the ulnar or jugular veins into 2-ml heparinized vacuum tubes. Because samples were collected during the execution of several disparate projects, the methods of handling and storage were not uniform; we describe the differences below. However, all samples met the minimum criteria for collection and storage to allow detection of the RE virus proviral DNA using the polymerase chain reaction (PCR).

During 1998, 1999, and 2000 we collected blood samples from 354 Greater Prairie-Chickens (231 males and 123 females) in seven states (Table 1). The greater number of males sampled reflects the fact that most trapping occurred on leks, where males predominate. Samples were obtained from Greater Prairie-Chickens on $\geq$38 trap sites, although in some cases these locations were $<$1 km apart.

We collected samples from four of the states, Wisconsin, Minnesota, North Dakota, and Nebraska, during 1998 and 1999 (Table 1) between July and August each year. We kept the samples on ice until centrifuged. The cellular fraction of each sample was frozen

TABLE 1. The number of samples collected from Greater and Lesser prairie-chickens, by state, year, and sex (M or F). The number of samples corresponds to the number of individual birds sampled except for Okahoma, where 120 Greater Prairie-Chickens and 95 Lesser Prairie-Chickens were sampled (34 birds were sampled twice) and New Mexico where 74 Lesser Prairie-Chickens were sampled, with five being sampled twice.

	Greater Prairie-Chicken						Lesser Prairie-Chicken			
	1998		1999		2000		1999		2000	
State	M	F	M	F	M	F	M	F	M	F
Wisconsin[a]	12	6	18	2						
Minnesota[b]			18	10						
North Dakota[c]			10	8						
Nebraska[d]	4	8								
Missouri[e]			31	2						
Kansas[f]			30	48			3			
Oklahoma[g]	14	11	73	26	21	2	42	3	37	20
New Mexico[h]							22	5	34	18
Total	30	25	180	96	21	2	67	8	71	38

[a] Adams, Marathon, Portage, and Wood counties.
[b] Clay, Norman, and Polk counties.
[c] Grand Forks County.
[d] Garfield, Loup, and Rock counties.
[e] Barton, Dade, Pettis, and St. Clair counties.
[f] Greenwood, Morton, Lyon, and Waubansee counties.
[g] Beaver, Ellis, Harper, and Osage counties.
[h] Roosevelt County.

at −18°C until August 1999, when they were shipped to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) for analysis.

We collected samples from the remaining three states, Kansas, Missouri, and Oklahoma, during 1998, 1999, and 2000 (Table 1). Although it is unlikely, a small number of samples taken in Missouri may have been from birds transplanted from Kansas in 1995. We collected samples twice 1–13 months apart from 27 individual Greater Prairie-Chickens in Oklahoma to determine seasonal changes in prevalence of the disease (if it were present).

We obtained most samples from March through May of each year, although some samples were collected throughout the year. The samples were frozen (−70°C or −18°C) whole in heparinized tubes, except for 17 samples from Oklahoma during 1998, which were kept refrigerated (4°C) for 3–4 months before being shipped to the TVMDL for analysis.

We collected samples from 184 Lesser Prairie-Chickens (138 males and 46 females) in Kansas, Oklahoma, and New Mexico at 17 trap sites. The samples were collected throughout 1999 and 2000 (Table 1), although the majority were obtained from March through May of each year. Except for 19 samples from New Mexico collected during 1999, all samples were frozen (−70°C or −18°C) whole in heparinized tubes ≤4 h of being collected and maintained frozen until shipped to the TVMDL. The 19 New Mexico samples were refrigerated for three months before shipment to the lab for analysis. As with Greater Prairie-Chickens, we collected samples twice at least one month apart from seven individual Lesser Prairie-Chickens in Oklahoma and five from New Mexico to explore seasonal changes in prevalence of the disease.

All samples were shipped on dry ice to the TVMDL for analysis during July of each year. They were tested for the presence of integrated proviral DNA of the viruses causing RE using PCR methods previously described (Aly et al. 1993, Davidson et al. 1995). The TVMDL personnel were experienced in use of this technique with prairie-chicken samples.

RESULTS

Of the 538 samples, only two were positive by PCR for RE virus proviral DNA. Both were from male Greater Prairie-Chickens sampled in Osage County, Oklahoma, during 1998. Because these two samples had been refrigerated, not frozen, they had degraded to the point where virus isolation (as opposed to detection of the integrated proviral DNA) could not be performed to verify the presence of the active virus.

DISCUSSION

The positive results on two Greater Prairie-Chickens in 1998 from Oklahoma add to the few reports of reticuloendotheliosis viruses in free-ranging galliforms (Ley et al. 1989, Hayes et al. 1992, Drew et al. 1998). Samples collected from an additional seven Lesser Prairie-Chickens in the Texas panhandle during 1997 and tested at the TVMDL also were negative for RE (M. J. Peterson pers. comm.). Given only two positives of more than 500 samples,

RE appears to be uncommon and may not to be a major threat to prairie-chicken populations.

The occurrence of two positive birds from 25 collected in Osage County, Oklahoma, in 1998, but no other positive birds from other areas or other years invites explanation. Among the possibilities, not mutually exclusive, are (1) the two positive results could be false positives; (2) the virus was present in prairie-chickens during 1998 but not in subsequent years; (3) the disease may enter the prairie-chicken population from a reservoir species, and suitable conditions, such as a high vector population, may not occur in all years; and (4) the disease may have been present but not detected due to the timing of sampling. Further data are needed to evaluate the likelihood of these possibilities. In addition, it could be useful to investigate whether individual prairie-chickens have antibodies to the RE viruses. The detection of antibodies may help to identify birds that have been exposed to the virus but carry proviral copies at numbers below threshold sensitivity of the PCR test, and may provide information about past exposure to the disease.

Although our survey demonstrated at best a low prevalence of RE, because of the potentially serious consequences of RE described in Drew et al. (1998), the possible presence of RE in the wild populations of prairie-chickens should be carefully considered in any future relocation and reintroduction efforts.

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LITERATURE CITED

ALY, M. M., E. J. SMITH, AND A. M. FADLY. 1993. Detection of reticuloendotheliosis virus infection using polymerase chain reaction. Avian Pathol. 22:523–554.

BAGUST, T. J. 1993. Reticuloendotheliosis virus. Pp. 437–454 *in* Virus infections of birds (J. B. McFerran and M. S. McNulty, Eds.). Elsevier Science Publishers, New York.

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 Pathol. 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. J. Wildl. Dis. 34:783–791.

FRIEND, M., R. G. MCCLEAN, AND F. J. DEIN. 2001. Disease emergence in birds: challenges for the Twenty-first Century. Auk 118:290–303.

GAUNT, A. S., L. W. ORING, K. P. ABLE, D. W. ANDERSON, L. F. BAPTISTA, J. C. BARLOW, AND J. C. WINGFIELD. 1999. Guidelines for the use of wild birds in research, 2nd ed. Ornithological Council, Washington, D.C.

HAYES, L. E., K. A. LANGHEINRICH, AND R. L. WITTER. 1992. Reticuloendotheliosis in a Wild Turkey (*Meleagris gallopavo*) from coastal Georgia. J. Wildl. Dis. 28:154–158.

LEY, D. H., M. D. FICKEN, D. T. COBB, AND R. L. WITTER. 1989. Histomoniasis and reticuloendotheliosis in a Wild Turkey (*Meleagris gallopavo*) in North Carolina. J. Wildl. Dis. 25:262–265.

WESTEMEIER, R. L. AND S. GOUGH. 1999. National outlook and conservation needs for Greater Prairie-Chickens. Pp. 169–187 *in* The Greater Prairie-Chicken: a national outlook (W. D. Svedarsky, R. H. Hier, and N. J. Silvy, Eds.). Univ. of Minnesota Misc. Publ. 99-1999, St. Paul.

WITTER, R. L. 1997. Reticuloendotheliosis. Pp. 467–484 *in* Diseases of poultry, 10th ed. (B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, Eds.). Iowa State Univ. Press, Ames.