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NORTHERN BOBWHITES AS DISEASE INDICATORS FOR THE ENDANGERED ATTWATER'S PRAIRIE CHICKEN

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ABSTRACT: Because of limited access to the endangered Attwater's prairie chicken (*Tympanuchus cupido attwateri*), we used a related species, the northern bobwhite (*Colinus virginianus*), as a surrogate for disease evaluation. Free-living northern bobwhites (n = 62) on the Attwater Prairie Chicken National Wildlife Refuge (near Eagle Lake, Texas, USA) were examined during spring and fall 1993 for helminthic endoparasites and specific antibodies against the infectious agents responsible for nine infectious diseases. *Trichostrongylus cramae*, *Raillietina* sp., and *Strongyloides avium* were collected from 97, 44, and 32% of northern bobwhites examined, respectively. *Dispharynx nasuta* and *Syngamus trachea* also were found. No gross lesions due to parasites were observed. Specific antibody to *Pasteurella multocida* was found in 3 of 53 plasma samples. It is possible that potentially pathogenic species such as *P. multocida*, *T. cramae*, and *D. nasuta* could threaten sympatric Attwater's prairie chickens.

Key Words: Attwater's prairie chicken, *Colinus virginianus*, infectious disease, northern bobwhite, parasite, serologic survey, *Tympanuchus cupido attwateri*.

INTRODUCTION

The Attwater's prairie chicken (*Tympanuchus cupido attwateri*) is an endangered prairie grouse endemic to the coastal prairies of Texas (USA). It was listed as endangered in 1967 and is predicted to become extinct by the year 2000 if current trends continue (U.S. Fish and Wildlife Service [USFWS], 1993; Peterson and Silvy, 1996). By spring 1997, approximately 58 individuals, including those released from captivity, remained in three populations (USFWS, unpublished data).

Large numbers of waterfowl winter on and around the Attwater Prairie Chicken National Wildlife Refuge (APCNWR; Eagle Lake, Texas, USA). Managers have speculated that these waterfowl may serve as a disease reservoir. Avian cholera, caused by *Pasteurella multocida*, has been diagnosed in waterfowl along the Texas gulf coast, including those on APCNWR (USFWS, unpublished data). This disease has been documented for red grouse (*Lagopus lagopus*) (Jennings, 1955) and ruffed grouse (*Bonasa umbellus*) (Green and Shillinger, 1936), so it is reasonable to assume that Attwater's prairie chickens also could be infected. Furthermore, the nematode *Trichostrongylus tenuis* has been responsible for the red grouse mortality in the United Kingdom (Leslie and Shipley, 1912; Potts et al., 1984; Hudson, 1986) and can influence red grouse population dynamics (Potts et al. 1984; Hudson et al., 1992). This nematode is common in geese (McDonald, 1969) and has been found in wild snow geese (*Chen caerulescens*) and white-fronted geese (*Anser albifrons*) near the APCNWR (Purvis et al., 1997). It is possible that *T. tenuis* could be transmitted to Attwater's prairie chickens when wintering geese defecate on Attwater's prairie chicken feeding and lekking areas. Whether sympatric northern bobwhites (*Colinus virginianus*) might serve as disease reservoirs for the endangered Attwater's prairie chicken is unknown.

Some infectious agents are thought to limit and/or regulate avian populations (Levine and Goble, 1947; Bendell, 1955; Hudson et al., 1992). However, there has been only one survey of the infectious diseases of Attwater's prairie chickens; Peter-

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son et al. (1998) examined fecal samples and necropsied five opportunistically collected carcasses. *Dispharynx nasuta* and *Trichostrongylus cramae* were found for one of three and eight of nine individuals, respectively, and specific antibody to *P. multocida* was found in 4 of 27 birds. However, the scope of their study was limited due to the endangered status of the Attwater's prairie chicken, and many important questions could not be addressed.

In this study we used parasitologic and serologic methods to examine infectious diseases in a population of northern bobwhites sympatric with Attwater's prairie chickens. Using a surrogate species allowed us to employ methods that otherwise would have been prohibited due to the protected status and limited numbers of Attwater's prairie chickens. Peterson (1996) used parasite surveys of other prairie grouse to predict the helminthic parasites of Attwater's prairie chickens. Surrogate species also have been used in studies on California condors (*Gymnogyps californianus*) (Collar et al., 1992) and black-footed ferrets (*Mustela nigripes*) (Powell et al., 1985). Because of taxonomic, habitat use, and behavioral similarities, sympatric populations of northern bobwhites are suitable surrogates for the Attwater's prairie chicken.

MATERIALS AND METHODS

During March and April 1993, 53 northern bobwhites were collected from the APCNWR, (Colorado County, Texas; 29°40′N, 96°20′W) using baited walk-in funnel traps. Traps were placed in areas of known northern bobwhite activity and baited 1 to 7 days before trapping began. All northern bobwhites captured were collected and traps removed after >5 birds were taken at a trap site. Nine additional bobwhites were collected with a shotgun at the same location during November, 1993.

Spring birds were placed in holding cages and processed at the end of the day; fall birds were placed in plastic bags after retrieval and stored on ice until being processed each evening. The body weight, sex, and age of each bird was recorded (Koerth et al., 1991). For spring birds, blood samples were taken via jugular puncture, then each bird was humanely killed and frozen in individual plastic bags; fall birds were frozen after weighing. Plasma was frozen at −20 C pending serologic evaluation at the Texas Veterinary Medical Diagnostic Laboratory (TVMDL; College Station, Texas, USA).

Plasma samples were tested for specific antibody against the avian influenza, infectious bronchitis, and Newcastle's disease viruses using microhemagglutination-inhibition tests (Beard and Wilkes, 1973); *Chlamydia psittaci* using an elementary body agglutination test (Grimes et al., 1994); *Mycoplasma gallisepticum* and *M. synoviae* using plate antigen tests (Veterinary Services, 1993); and *Salmonella pullorum* and *S. typhimurium* using tube agglutination tests (Veterinary Services, 1993). Testing for specific antibody to *P. multocida*, using an enzyme-linked immunosorbent assay (ELISA) (FlockChek Anti-Pm, Idexx Corp., Portland, Maine, USA), was conducted by the Diagnostic Services Laboratory (DSL) (Poultry Disease Research Center, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA). Although this commercial ELISA used anti-domestic chicken conjugate instead of anti-northern bobwhite conjugate, DSL personnel have found it satisfactory for several avian species including turkeys and waterfowl (S. G. Thayer, unpublished data). For this reason, our results should be conservative.

Intact viscera and their contents were removed and examined under a dissecting microscope. Nematodes were examined in glycerin jelly mounts. Cestodes were stained in Semichon's acetocarmine, sequentially dehydrated in ethanol, cleared in xylene, and mounted in Kleermount (Carolina Biological Supply Company, Burlington, North Carolina, USA). Reference specimens of *T. tenuis* and *T. cramae* from various hosts in the U.S. National Parasite Collection (Beltsville, Maryland, USA) were compared with our specimens and recent descriptions of *Trichostrongylus* spp. from birds (Durrette-Desset et al., 1993). Representative specimens were deposited in the U.S. National Parasite Collection (Biosystematics and National Parasite Collection Unit, U.S. Department of Agriculture, Beltsville, Maryland, USA; accession number(s) were 86971 for *D. nasuta*, 84210 and 86964 for *Strongyloides avium*, 84207 for *Syngamus trachea*, and 86973–86974 for *T. cramae*). Representative specimens also were deposited in the University of Nebraska State Museum (Systematics Research Collections, Division of Parasitology, Harold W. Manter Laboratory, Lincoln, Nebraska, USA; accession number(s) were 37989 for *S. avium* and 37987 for *S. trachea*).

Cestode prevalence and intensity (as defined

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by Margolis et al., 1982) were determined by counting scoleces; if only proglottids were found, intensity was counted as one, regardless of the number of proglottids present. The species, sex, and parasite portion (whole, anterior, center, posterior) of each mounted nematode was recorded and the minimum number of nematodes computed. Mean prevalence and intensity (±1 standard deviation) were calculated for each species and the total number of nematodes and helminths were recorded by host sex and age. Independence of prevalence by sex and age was tested using chi-square contingency tables (SAS Institute Inc., 1988b). Because intensities were non-normally distributed, differences due to sex and age were tested using the Wilcoxon rank-sum test (SAS Institute Inc., 1988a). Relationships between host mass and parasite intensity were tested using Spearman's rank-order correlation test (SAS Institute Inc., 1988b). All statistical tests were conducted at the $P \leq 0.05$ level of significance.

RESULTS

All plasma samples tested were negative for specific antibodies to the avian influenza and Newcastle's disease viruses, *M. gallisepticum, M. synoviae, S. pullorum,* and *S. typhimurium* ($n = 53$), *C. psittaci* ($n = 49$), and the infectious bronchitis virus ($n = 46$). Specific antibody to *P. multocida* was found for 3 of 53 birds.

Northern bobwhites were parasitized by one species of cestode and four species of nematodes (Table 1). *Trichostrongylus cramae, S. avium,* and *Raillietina* sp. were found in 60 of 62 (97%), 20 of 62 (32%), and 27 of 62 (44%) birds, respectively. *Raillietina* sp. could not be identified to species due to specimen damage caused by freezing. Prevalences of all endoparasites were independent of age and sex ($P \geq 0.31$ and 0.14, respectively). The prevalence of *S. avium* was dependant upon sex and age interactions, with more adult females parasitized than adult males and juvenile females ($P = 0.002$ and 0.03, respectively), while more juvenile than adult males were parasitized ($P = 0.01$).

Strongyloides avium, T. cramae, and *Raillietina* sp. (Table 1) comprised 19, 50, and 30%, respectively, of all helminths collected. No significant differences ($P \geq 0.23$) in intensity were detected due to age alone. Females had higher intensities of total nematodes and total helminths than males ($P = 0.004$ and 0.02, respectively). Adult female northern bobwhites had higher intensities of both total nematodes and total helminths than did adult males ($P = 0.01$ and 0.02, respectively), while juvenile males had a higher mean intensity of *Raillietina* sp. than did adult males ($P = 0.02$). Host mass and parasite intensity were not significantly related ($P \geq 0.10$).

DISCUSSION

How easily *P. multocida* can be transmitted from waterfowl, northern bobwhites, or the environment to Attwater's prairie chickens is unknown. However, evidence of *P. multocida* exposure in both northern bobwhites from the APCNWR and Attwater's prairie chickens from two of the three remaining populations (Peterson et al., 1998) has led managers of the APCNWR to implement strategies designed to limit exposing Attwater's prairie chickens to this infectious agent.

Trichostrongylus cramae, as recently distinguished from *T. tenuis* by Durette-Desset et al. (1993), has been found in northern bobwhites throughout the southern United States (Cram et al., 1931; Kellogg and Prestwood, 1968; Moore et al., 1986; Demarais et al., 1987). Prevalence often reaches 100%, with highest intensities occurring in the late winter (Davidson et al., 1980; Moore et al., 1986). Although Davidson et al. (1991) found that intensities >200 were related to decreased body mass in northern bobwhites, this parasite is not considered pathogenic (Freehling and Moore, 1993) or regulatory for northern bobwhite populations (Davidson et al., 1982). Peterson et al. (1998) first demonstrated *T. cramae* in prairie grouse, finding this nematode in all four Attwater's prairie chickens necropsied that still retained the cecum. This, along with their finding of *T. cramae* eggs in cecal voids from all remaining populations, suggests that *T. cramae* is widespread in Attwater's prairie

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TABLE 1. Mean intensity, range, and prevalence of helminths from northern bobwhites collected during 1993 at the Attwater Prairie Chicken National Wildlife Refuge, Colorado County, Texas.

	Immature quail			Adult quail			
	Males	Females	All	Males	Females	All	Totals
Number examined	10	11	21	24	17	41	62
Dispharynx nasuta	— (—) 0[a]	— (—) 0	— (—) 0	1 (1) 4	2.5 ± 0.7 (2–3) 12	2 ± 1 (1–3) 7	2 ± 1 (1–3) 5
Raillietina sp.	59 ± 90 (9–218) 50	54 ± 99 (1–250) 55	56 ± 90 (1–250) 52	22 ± 53 (1–153) 33	22 ± 22 (1–72) 47	22 ± 39 (1–153) 39	36 ± 66 (1–250) 44
Strongyloides avium	4 ± 4 (1–10) 50	21 ± 4 (18–23) 18	9 ± 9 (1–23) 33	21 ± 25 (2–50) 13	50 ± 58 (1–162) 59	43 ± 53 (1–162) 32	31 ± 46 (1–162) 32
Syngamus trachea	— (—) 0	— (—) 0	— (—) 0	— (—) 0	1.5 ± 0.7 (1–2) 12	2 ± 1 (1–2) 5	2 ± 1 (1–2) 3
Trichostrongylus cramae	13 ± 4 (1–42) 100	32 ± 28 (1–99) 100	23 ± 24 (1–99) 100	24 ± 28 (1–126) 96	37 ± 69 (2–286) 94	29 ± 48 (1–286) 95	27 ± 41 (1–286) 97
Unknown nematode	1 (1) 30	2 (2) 9	1 ± 1 (1–2) 19	2 ± 1 (1–2) 8	1 (1) 18	1 ± 0 (1–2) 12	1 ± 0 (1–2) 15
Total nematodes	15 ± 15 (1–44) 100	36 ± 33 (1–122) 100	26 ± 27 (1–122) 100	27 ± 29 (1–126) 96	69 ± 75 (9–287) 94	44 ± 56 (1–287) 95	38 ± 49 (1–287) 97
Total helminths	45 ± 80 (1–262) 100	65 ± 91 (3–294) 100	56 ± 84 (1–294) 100	35 ± 39 (1–157) 96	80 ± 77 (9–287) 94	53 ± 61 (1–287) 95	54 ± 69 (1–294) 97

[a] Mean intensity ± standard deviation (range) prevalence in %.

chickens. However, the importance of this parasite to Attwater's prairie chicken populations is unknown.

Dispharynx nasuta is a common parasite of galliform and passeriform birds (Goble and Kutz, 1945) and has caused significant mortality of pen-raised northern bobwhites (Kellogg and Prestwood, 1968). Although *D. nasuta* is generally thought to be unimportant to the northern bobwhite (Davidson et al., 1991), it apparently can negatively impact blue grouse (*Dendragapus obscurus*) (Bendell, 1955) and ruffed grouse (Goble and Kutz, 1945; Edminster, 1947) individuals and populations. In contrast, Harper et al. (1967) did not consider *D. nasuta* pathogenic for the adult greater prairie chickens (*T. cupido pinnatus*) they necropsied in Kansas. Although Peterson et al. (1998) found *D. nasuta* in the proventriculus of an adult Attwater's prairie chicken, its pathogenicity for this host species is unknown. Because *D. nasuta* uses isopods as intermediate hosts (Cram et al., 1931), it is likely to be more prevalent in the primarily insectivorous Attwater's prairie chicken chicks than in adults.

We found three parasites in addition to those observed by Peterson et al. (1998). The common gapeworm, *S. trachea*, has caused mortality in captive ring-necked pheasants (*Phasianus colchicus*) (Wehr, 1971), but has not been reported previously from prairie grouse. *Strongyloides avium* has not been widely reported in northern bobwhites. Davidson et al. (1980) found higher prevalence of *S. avium* in adults than for juveniles (22 vs. 5%), but equal intensities. Although we found high *S. avium* intensities, these results are difficult to interpret. Even though *S. avium* has not been reported from prairie grouse, it is reasonable to assume Attwater's prairie chickens could serve as hosts because of its broad host range. *Raillietina* spp. are common in northern bobwhites and six species have been reported (Scott, 1985). Cestodes are not generally considered pathogenic. However, Cram et al. (1931) reported that high intensity infections of *R. tetragona* caused partial intestinal paralysis in northern bobwhites. Although we found high intensities, no gross lesions were observed. Four species of cestodes have been reported from prairie grouse (Peterson, 1996), including *R. variabilis* and *R. centrocerci*.

The use of the northern bobwhite as a surrogate for the Attwater's prairie chicken provided useful information, particularly when used in conjunction with the study of Peterson et al. (1998). We found that sympatric northern bobwhites had been exposed to all infectious agents found by Peterson et al. (1998). We also found three additional parasites in northern bobwhites that have broad host ranges and probably could infect Attwater's prairie chickens. Unlike Peterson et al. (1998), we were able to collect sufficient numbers of birds to estimate parasite prevalence and intensity for this population. Experiments using captive greater prairie chicken chicks, another surrogate, would be necessary to determine the pathogenicity of these infectious agents for prairie chickens.

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