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SEROLOGIC AND PARASITOLOGIC SURVEY OF THE ENDANGERED ATTWATER'S PRAIRIE CHICKEN

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ABSTRACT: Because conservation biologists have postulated that infectious diseases may have potentiated the endangerment of the Attwater's prairie chicken (*Tympanuchus cupido attwateri*), free-living prairie chickens were surveyed from all remaining populations for helminthic endoparasites and antibody against the etiological agents of nine infectious diseases. Samples from 4 of 27 adult males were positive for anti-*Pasteurella multocida* antibody. All other serologic tests were negative (n = 19). We identified *Dispharynx nasuta*, a parasite previously associated with disease in other grouse from North America, in one of three adult Attwater's prairie chickens examined. Evidence of *Trichostrongylus cramae* was found for eight of nine suitable samples, which represents the first report of this parasite in prairie grouse. The mean intensity of *T. cramae* in Attwater's prairie chicken was 1,019.3 (Range = 3-1,906; n = 3). Further work is needed to determine whether *P. multocida*, *T. cramae*, or *D. nasuta* are detrimental to Attwater's prairie chicken populations. If so, conservation biologists could reduce the prevalence and incidence of these parasites and potentially gain more time to address the habitat conditions thought to be the ultimate cause of population declines.

Key words: Attwater's prairie chicken, Dispharynx nasuta, parasite survey, Pasteurella multocida, serologic survey, Trichostrongylus cramae, Tympanuchus cupido attwateri.

INTRODUCTION

The U.S. Fish and Wildlife Service (USFWS) listed the Attwater's prairie chicken (Tympanuchus cupido attwateri) as endangered in 1967 when approximately 1,070 birds remained in the wild (USFWS. 1983; Peterson and Silvy, 1996). Historically, this prairie grouse occupied an estimated 2.4 million ha of coastal prairie from southwestern Louisiana (USA), south, to at least the Nueces River in Texas (USA) (Lehmann, 1941; Peterson and Silvy, 1996). Most researchers maintain that conversion of coastal prairie to agricultural, commercial, and urban uses ultimately caused the long-decline in Attwater's prairie chicken numbers and the elimination of this species from Louisiana and most of Texas (Lehmann, 1941; Cogar et al., 1977; USFWS, 1983). By spring 1996, only 42 individuals remained in three isolated populations occurring in Colorado, Galveston, and Refugio counties, Texas (USFWS, unpubl. data). Although the quantity of suitable habitat is much less than in the previous century, there appears to be substantially more than required to support 42 prairie chickens (Peterson and Silvy, 1996). Because of the risk of stochastic extinction (Gilpin and Soulé, 1986) in these small, isolated populations, conservation biologists need a clear understanding of the proximate causes of the short-term fluctuations observed in Attwater's prairie chicken numbers (Peterson and Silvy, 1994, 1996).

Managers surmise that several factors, besides habitat degradation, might have contributed to the endangerment of the Attwater's prairie chicken—including infectious disease (USFWS, 1993). Although some factors thought to influence the dynamics of Attwater's prairie chicken populations have been examined (Horkel et al., 1981; Lutz et al., 1994; Peterson and Silvy, 1994; Morrow et al., 1996), the importance of infectious agents is unknown. We found no published records of disease surveys of free-living Attwater's prairie chickens other than Lehmann's (1941, p. 36) comment that "no evidence of disease or heavy parasitism was found in autopsies made on 13 prairie chickens, and no evidence of any unhealthful condition was observed among hundreds of birds in the field." However, between November 1967 and January 1968, 26 Attwater's prairie chickens were captured at Ellington Air Force Base (Texas, USA) and brought into captivity in a poultry facility. Eight birds died of blackhead disease, five of aspergillosis, one of Tetrameres americanus infection, two of Trichostrongylus sp., and one of a non-specified (but thought to be bacterial) enteritis (Kubena, 1969; Brownlee, 1970, p. 3). Although these birds may have contracted these infectious agents in the poultry facility, the fact remains that Attwater's prairie chickens can contract and succumb to these macro- and microparasites.

Peterson (1996) found a gradient among the helminthic endoparasite communities of prairie grouse collected from five North American ecological divisions (Bailey, 1978) that could be explained by precipitation and winter temperature. This spatial analysis predicted that Attwater's prairie chickens might have helminth communities similar to those of greater prairie chicken (T. cupido pinnatus) populations surveyed in Illinois, Kansas, and Missouri (USA). Two parasite species associated with disease in grouse in North America, Dispharynx nasuta and Heterakis gallinarum, were found. Because of precipitation patterns in the Texas Gulf coast, Peterson (1996) also predicted that Attwater's prairie chickens might be infected with helminths not found during other parasite surveys of prairie grouse. These hypotheses have yet to be evaluated.

We surveyed free-living Attwater's prairie chickens from all remaining populations for endoparasitic helminths and specific antibody against the etiological agents of nine infectious diseases. We used these results to determine whether individuals harbor infectious agents known to cause disease in grouse and whether Peterson's (1996) hypotheses are consistent with empirical data. Unfortunately, because so few Attwater's prairie chickens remain, many traditional survey methods could not be used, thus constraining our methodology. We detail these constraints as we explain our methods.

MATERIALS AND METHODS

Our permits allowed us to collect blood only from males and required us to release all individuals immediately at point of capture. Attwater's prairie chickens were captured, using drop nets (Silvy et al., 1990), during spring 1992 and 1993 on booming grounds at the Attwater Prairie Chicken National Wildlife Refuge (NWR) in Colorado County (29°42'N, 96°17'W), the Rio Ranch in Galveston County (29°28'N, 94°57'W), and the Williams Ranch in Refugio County (28°33'N, 97°03'W), Texas. We banded each captured grouse using both numbered aluminum and colored plastic leg bands, recorded the sex and weight of each bird, and classified each individual as either juvenile or adult using Ammann's (1944) outer primary technique. A 2 ml blood sample was collected via jugular puncture from all males with a heparinized 3-ml syringe and a 1 inch, 22 gauge needle. Blood samples (n = 19) were placed in sterile vacutainer tubes and stored on ice during transport to the laboratory.

All heparinized blood samples were centrifuged, the plasma placed in sterile vials, and the samples stored at -20 C pending serologic analysis. We submitted the plasma samples to the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas, USA) for serological testing for specific antibody against Salmonella typhimurium and S. pullorum using tube agglutination tests (Veterinary Services, 1993, pp. 45–50); Mycoplasma gallisepticum and M. synoviae using plate antigen tests (Veterinary Šervices, 1993, pp. 54–55), Chlamydia sp. using an elementary body agglutination test (Grimes et al., 1994); and the Newcastle disease, infectious bronchitis, and avian influenza viruses using microhemagglutination-inhibition tests (Beard and Wilkes, 1973).

Serological testing for specific antibody to *Pasteurella multocida*, using an enzyme-linked immunosorbent assay (ELISA) (FlockChek Anti-Pm, Idexx Corp., Portland, Maine, USA), was conducted by the Diagnostic Services Laboratory of the Poultry Disease Research Center (College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA). The 19 plasma samples collected during this study, and eight additional samples collected during 1987, were tested. We considered a sample positive for exposure to *P. multocida* if the ratio of the optical density of the sample to the positive control was >0.2, as suggested by the manufacturer. This commercial ELISA used anti-domestic chicken, rather than anti-Attwater's prairie chicken, conjugate. Anti-chicken conjugate performs satisfactorily for several avian species, such as turkeys and waterfowl, in addition to the domestic chicken (S. G. Thayer, unpublished, data).

Because of their low numbers and endangered status, we could not collect Attwater's prairie chickens, conduct necropsies, and perform thorough parasitologic examinations. We collected fresh Attwater's prairie chicken feces from booming grounds each morning after trapping was completed and pooled all specimens from a single ground. We also attempted to locate as many roost sites as possible and collect fresh cecal feces (prairie chickens typically do not roost on booming grounds). We put all specimens in plastic bags and placed them on ice for transport. Both examinations for nematode eggs (Foreyt, 1990) and larval culture (Craig, 1993) were begun on each fecal sample immediately upon returning from the field. If unidentified larvae were found, domestic chicks <1 wk of age were inoculated per os with approximately 25 infectious stage larvae each. Inoculated and control chicks were killed at 1 wk post-inoculation by cervical dislocation, and biweekly thereafter, and the complete gastrointestinal contents examined for helminthic endoparasites.

We also opportunistically obtained data from Attwater's prairie chickens found dead during unrelated management activities. In a project designed to locate prairie-chicken nests, one female died during trapping and was submitted to the Schubot Exotic Bird Heath Center (Texas A&M University, College Station, Texas, USA) for necropsy. Four additional Attwater's prairie chickens were made available directly to us for necropsy: two radio-marked birds killed by hawks and two that died when brought into captivity. Intact viscera (where present) from these four birds were removed and each gastrointestinal organ separately examined. After external examination, each organ was cut lengthwise, the contents place in a petri dish, and both the contents and intestinal walls examined for helminths under a dissecting microscope. Nematodes were examined in glycerin jelly mounts. We found no cestodes, trematodes, or acanthocephalans.

The species, sex, and parasite portion (whole, anterior, center, posterior) of each mounted nematode was recorded and the minimum number of nematodes computed. Parasites were identified according to Yamaguti (1961) and recent descriptions of Trichostrongylus spp. from wild birds (Durette-Desset et al., 1993). Specimens of Trichostrongylus sp. also were compared with reference specimens of T. tenuis and T. cramae from a variety of hosts in the U.S. National Parasite Collection (Beltsville, Maryland, USA). Representative specimens of all helminths 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 84212-84213 and 84408-84411 for T. cramae, and 84208 for D. nasuta). 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 37991, 37993, 38151 for T. cramae, and 37988 for D. nasuta).

RESULTS

All 19 plasma samples (five from Colorado, 10 from Galveston, and four from Refugio counties) were negative for specific antibody against *S. typhimurium*, *S. pullorum*, *M. gallisepticum*, *M. synoviae*, *Chlamydia* sp., and the Newcastle disease, infectious bronchitis, and avian influenza viruses. Of 27 samples tested for specific antibody against *P. multocida*, 3 of 13 collected on the Attwater Prairie Chicken NWR (2 in 1987; 1 in 1993) and 1 of 10 (1992) collected in Galveston County were positive.

One adult female, collected immediately after death (March 1992) at the Attwater Prairie Chicken NWR by refuge personnel and submitted to the Schubot Exotic Bird Heath Center, had sections of a small nematode in the cecal lumen between the low mucosal plicae and occasionally in the gland crypts. No other histopathologic changes relating to parasitism were noted. Trichostongylid eggs were present in the small section of this bird's cecum retained by the pathologist. This section of the cecum was submitted to us. Larvation was successful and one to two adult T. cramae were collected in four of the seven inoculated chicks. This was the

first demonstration of *T. cramae* from freeliving prairie grouse.

We found no parasite eggs or larvae in 16 pooled fecal samples (each representing 2 to 5 individuals) collected from booming grounds in Galveston County and the Attwater Prairie Chicken NWR. During the 1992 field season, one cecal fecal sample was collected from a roost on the Rio Ranch, Galveston County, Texas. No eggs or larvae were found. During 1993, trichostrongylid eggs were found in one cecal void from a roost in Refugio, two from Austin, and one from Colorado County. Parasite larvae were cultured from the samples collected in Refugio and Austin counties. These trichostrongylid larvae appeared identical to those determined to be T. cramae by domestic chick subinoculation. No larvae were cultured from the Colorado County sample, possibly due to its age.

Three of the adult Attwater's prairie chickens we necropsied were infected with T. cramae in the cecum, while one was infected with D. nasuta in the proventriculus. These are new host records for D. nasuta and T. cramae. The cecum and ileum of the male captured on the Attwater Prairie Chicken NWR in Colorado County (April 1994) contained 1,122 and 27 T. cramae, respectively. The proventriculus was not available for examination. The cecum of the male from Austin County (April 1994) contained 1,906 T. cramae. Thirteen D. nasuta were found in the proventriculus of an adult female found dead in Refugio County (April 1994). Although approximately 33% of this bird's cecum was missing, three T. cramae were found in the remaining portion. We also necropsied an adult female killed by an avian predator in Galveston County during March, 1993. Unfortunately, the portion of the gastrointestinal tract distal to the proventriculus was not available for examination. No parasites were seen in the proximal portion. Therefore, T. cramae was found in three of three, and D. nasuta in one of three, suitable necropsy specimens.

DISCUSSION

We found that free-living Attwater's prairie chickens are hosts for D. nasuta and T. cramae. This is consistent with the hypotheses Peterson (1996) derived, based upon the geographic prevalence of helminthic endoparasitism in prairie grouse. We also found evidence indicating that Attwater's prairie chickens were exposed to P. multocida. Because of the necessarily constrained nature of our survey, several questions remain unanswered. The lack of eggs or larvae in fecal samples, and negative serologic results derived from apparently healthy birds, does not imply that these birds were parasite free or that the infectious diseases for which we tested have not occurred in some individuals in these populations. Additionally, because we could not complete histopathologic examinations of the four prairie chickens we necropsied, we were unable to evaluate whether the helminths caused pathologic changes in these birds.

Although infectious disease has not been shown to regulate North American grouse populations, this fact does not imply that certain infectious agents do not play a limiting or regulatory role. Therefore, evidence of exposure to P. multocida and the identification D. nasuta and T. cramae in members of the remaining Attwater's prairie chicken populations has interested those managing these populations. Conservation biologists typically ask us what the significance of these agents might be to Attwater's prairie chicken individuals and populations, and how one might test these hypotheses. We incorporate these important issues in the remainder of this discussion.

Since the winter of 1988–89, avian cholera epizootics have occurred during many years in the coastal prairies of Texas and have killed thousands of wild geese and ducks sharing habitat with Attwater's prairie chickens (M. J. Peterson and D. S. Davis, unpublished data). How readily *P. multocida* can be transmitted from waterfowl or the environment to Attwater's prairie chickens, or whether other gallinaceous birds sharing this habitat, such as northern bobwhite (*Colinus virginianus*), can serve as effective reservoirs, is unknown. Nevertheless, managers of the Attwater prairie Chicken NWR have been sufficiently concerned about potential *P. multocida* exposure to systematically collect waterfowl found dead of avian cholera and incinerate them off refuge grounds.

Levine and Goble (1947) found that D. nasuta was responsible for significant disease and mortality in young ruffed grouse (Bonasa umbellus). Gross (1928) maintained that this parasite might have contributed to the extinction of the heath hen (T. cupido cupido). Similarly, Bendell (1955) found D. nasuta was an important cause of mortality in blue grouse (Dendragapus obscurus) chicks and a major factor in blue grouse population stability in his study area. If D. nasuta is as pathogenic for young Attwater's prairie chickens as it appears to be for ruffed and blue grouse chicks, it could contribute to the comparatively low number of juvenile Attwater's versus greater prairie chickens surviving per brood prior to brood breakup (Peterson and Silvy, 1996). Harper et al. (1967) did not consider D. nasuta detrimental to the greater prairie chicken populations they studied. One must note, however, that the viscera they examined were collected at hunter check stations in November, so no young birds were included in their sample. For this reason, they could not address the potential importance of a parasite that is typically pathogenic only to chicks. It would be informative to verify these early studies addressing the importance of D. nasuta to grouse population dynamics and chick health using modern epidemiological techniques.

Recently, Durette-Desset et al. (1993) described the *Trichostrongylus* sp. of northern bobwhite as *T. cramae* rather than *T. tenuis*. Freehling and Moore (1993) experimentally inoculated northern bobwhite with infective-stage *T. tenuis* larvae originating from red grouse (*Lagopus lagopus scoticus*) from northern England and *Trichostrongylus* sp. from northern bobwhite from Florida

(USA). This experiment confirmed that Trichostrongylus sp. of red grouse and northern bobwhite should be considered distinct species. Davidson et al. (1991) and Freehling and Moore (1993) found that T. cramae infections did not cause cecal lesions or inflammation in northern bobwhite and Davidson et al. (1982) found no evidence that T. cramae limits or regulates northern bobwhite populations. In red grouse, cecal lesions and inflammation caused by T. tenuis have long been recognized (Lovat, 1911; Watson et al., 1987). Moreover, high T. tenuis intensities were associated with reduced host fecundity and poor survival (Lovat, 1911; Potts et al., 1984; Hudson and Dobson, 1991; Hudson et al., 1992b). Experimental reductions in intensities demonstrated that increased T. tenuis intensities were associated with decreased body weight, adult survival, clutch size, egg hatchability, nesting success, and brood-rearing success in red grouse (Hudson, 1986; Shaw, 1990; Hudson et al., 1992a, b).

Interestingly, the intensity of T. cramae in Attwater's prairie chickens appears more similar to that seen for T. tenuis in red grouse in northern England and Scotland (Hudson, 1986; Shaw and Moss, 1989; Hudson et al., 1992a) than to T. cramae in northern bobwhite in the southeastern United States (Forrester et al., 1984; Moore et al., 1986; Davidson et al., 1991; Purvis, 1995). Because caecal length of Tympanuchus spp. and Lagopus spp. (both Tetraoninae) are similar, and much greater than that of the more distantly related northern bobwhite (Leopold, 1953), comparisons of Trichostrongylus sp. intensities of red grouse and Attwater's prairie chickens are not unreasonable.

Although we found *T. cramae* in members of all remaining Attwater's prairie chicken populations, we could not address whether *T. cramae* limits or regulates Attwater's prairie chicken populations. Experimental inoculation of captive greater prairie chicken chicks with infective-stage *T. cramae* larvae originating from Attwater's prairie chickens would be needed to address these questions. If T. cramae infections result in cecal lesions in prairie chickens similar to those shown detrimental to red grouse infected with T. tenuis, further research would be indicated. For example, experimental reductions in intensities in both captive greater and free-living Attwater's prairie chickens, similar to those conducted by Hudson (1986), Watson et al. (1987), Shaw (1990), and Hudson et al. (1992a) could be attempted. Such experiments would allow one to determine whether T. cramae leads to decreased body weight, adult survival, clutch size, and hatching, nesting, and brood rearing success in Attwater's prairie chicken as T. tenuis does in red grouse. Positive experimental evidence would help explain why Attwater's prairie chicken nesting success and number of chicks per brood prior to brood breakup are lower than those seen for the greater prairie chicken (Peterson and Silvy, 1996). Similarly, such evidence might lead conservation biologists to ask whether northern bobwhite populations, sharing habitat with Attwater's prairie chickens and having high T. cramae prevalence (60/62; Purvis, 1995), might serve as effective reservoir hosts. Experimental cross-species inoculation (using captive greater prairie chicken chicks) would be necessary to address this question. Conversely, if T. cramae infections in prairie chickens cause little or no pathogenic change, as observed in northern bobwhites, these additional studies would not be indicated and conservation biologists could better use resources elsewhere. A similar approach could be used to evaluate the potential importance of D. nasuta to Attwater's prairie chicken populations.

Because so few Attwater's prairie chickens remain, micro- or macroparasites that decrease productivity and/or increase mortality could increase the risk of stochastic extinction for these small, isolated populations (Gilpin and Soulé, 1986). If *P. multocida*, *T. cramae*, or *D. nasuta* are shown to be detrimental to Attwater's prairie chicken populations, conservation biologists could ad-

dress such conditions through management manipulation. Because the Attwater's prairie chicken is a lekking species, and so few remain in the wild, most individuals could be captured relatively easily early in the breeding season, treated, and immediately released at point of capture. Such comprehensive trapping efforts already have been used for other purposes during the last several springs. It also is possible that anthelmintic treated feed might effectively reduce the intensity of parasite infection. At any rate, if T. cramae or D. nasuta infections are found detrimental to Attwater's prairie chickens, then anthelmintic treatment could reduce the risk of stochastic extinction (Gilpin and Soulé, 1986). Similarly, if further research indicates avian cholera vaccine is safe and efficacious for greater prairie chickens in captivity, the few remaining Attwater's prairie chickens could be vaccinated. Biologists also could vaccinate individuals in the captive propagation program prior to releasing them. Such amelioration of proximate causes of the decline in Attwater's prairie chicken numbers could give conservation biologists additional time to address the habitat conditions thought to be the ultimate cause of the decline.

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