

HELMINTH INFECTIONS IN NORTHERN BOBWHITES (*COLINUS VIRGINIANUS*) FROM A LEGACY LANDSCAPE IN TEXAS, USA

Andrew C. Olsen^{1,2,3} and Alan M. Fedynich¹

¹ Caesar Kleberg Wildlife Research Institute, Texas A&M University–Kingsville, 700 University Boulevard, MSC 218, Kingsville, Texas 78363, USA

² Present address: Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, 2820 Southwest Campus Way, Corvallis, Oregon 97331, USA

³ Corresponding author (email: andrew.olsen@oregonstate.edu)

ABSTRACT: The Northern Bobwhite (*Colinus virginianus*) has declined across its range. The primary cause of this decline is thought to be habitat loss and fragmentation. However, there is speculation that factors such as parasites may play a role. South Texas recently was designated a Legacy Landscape of National Significance for Northern Bobwhite Conservation and is a region with some of the highest bobwhite densities in the US. Limited studies on bobwhite parasites have been conducted in this crucial landscape. We documented helminth parasites infecting bobwhites in South Texas, identified those that are known to be pathogenic to quail, documented pathologic responses to infection, and evaluated infections related to host intrinsic and extrinsic factors. We examined 209 bobwhites and found nine species of helminths including two known to cause tissue damage in bobwhites: *Tetrameres pattersoni* and *Oxyspirura petrowi*. The cecal nematode *Aulonocephalus pennula* was numerically dominant and had the greatest prevalence, intensity, and abundance. Prevalence and abundance of *A. pennula* were significantly greater in adult than juvenile bobwhites, whereas host sex was not an important factor. Prevalence of *A. pennula* was significantly greater during the 2012–13 hunting season than the 2013–14 season. The abundance of *A. pennula* also was significantly greater in bobwhites with greater mass within each age cohort. This research provides insight regarding the factors that influence helminth infections in bobwhites from South Texas and highlights the importance of broad-scale surveys when assessing helminth infections across large regions.

Key words: *Colinus virginianus*, helminths, Northern Bobwhite, parasites, pathogenic, Texas.

INTRODUCTION

The Northern Bobwhite (*Colinus virginianus*) is an economically and culturally important game bird that has experienced long-term population decline across its range in North America. Habitat loss and fragmentation generally are regarded as the causes of this decline (Brennan 1991). Most management and conservation efforts have focused on habitat development and improvement (Brennan et al. 2007; Hernández and Peterson 2007). South Texas holds approximately 4,000,000 ha of prime quail habitat and was designated a Legacy Landscape of National Significance for Northern Bobwhite Conservation by the National Bobwhite Conservation Initiative in July 2014 (Brennan 2014). The last parasitologic study in South Texas occurred over four decades ago (Demarais et al. 1987). Recently there has been speculation that disease and parasitism may be playing a

role in the regulation or decline of quail populations (Dunham et al. 2014). Little is known about parasitic infections in quails within Texas, leading to the call by Peterson (2007) for more research on bobwhite parasites and diseases in this state.

Herein we document helminth parasites infecting bobwhites in South Texas, identify those that are known to be pathogenic to quail, document pathologic responses to infection, and evaluate infections related to host intrinsic (age, sex, mass) and extrinsic factors (season of collection). Information obtained in this study is useful in assessing infection patterns and the impact of helminths on individual bobwhites and directing future research efforts in the region.

MATERIALS AND METHODS

Our study was conducted in South Texas, a region encompassing approximately 12,000,000

ha. This region generally consists of large ranches and minimal cropland with beef production as the primary land use (Hernández et al. 2002). The brush community that dominates the South Texas landscape primarily is composed of mesquite (*Prosopis glandulosa*), blackbrush (*Coleogyne ramosissima*), brasil (*Condalia hookeri*), and other thornscrub species (Inglis 1964). Grasses in South Texas such as bluestems (*Andropogon* spp. and *Schizachyrium* spp.), threeawns (*Aristida* spp.), and balsamscales (*Elionurus* spp.) are frequently used as nesting habitat by bobwhites (Lehmann 1946; Hernández and Guthery 2012). In addition, prickly pear (*Opuntia* spp.) provides important nesting cover for bobwhites (Silvy et al. 2007; Hernández and Guthery 2012). South Texas, as a whole, experiences a dynamic climate that is subject to periods of intense drought and heavy rainfall and flooding (Fulbright and Bryant 2003).

Bobwhites were donated by participating ranchers and lessees during two consecutive Texas quail hunting seasons (27 October 2012 to 24 February 2013 and 26 October 2013 to 23 February 2014). Cooperators were instructed to place each bird in an individual freezer bag, provide location and date, place in an ice cooler while in the field, and store in a freezer within 5 h of collection. We determined age (juvenile or adult) and sex of bobwhites during necropsy according to plumage characteristics (Leopold 1939). Masses (whole carcass weights) were determined with a digital scale. Bobwhites were obtained in accordance with permits from Texas Parks and Wildlife (SRP-0498-949) and authorizations by Texas A&M University-Kingsville Institutional Animal Care and Use Committee (2012-09-28A) and Texas A&M University-Kingsville Institutional Biosafety Committee (2012-11-28-IBC-C-A2).

We conducted thorough necropsies of each bobwhite specimen collected. The eyes, nictitating membrane, lacrimal ducts, intraorbital glands, nasal cavity, sinuses, and brain were dissected and searched for helminth parasites. The respiratory tract (trachea and lungs) and viscera (heart, kidneys, liver, gall bladder, esophagus, crop, proventriculus, gizzard, ceca, pancreas, spleen, small intestine, large intestine, cloaca, bursa [when present], and female reproductive tract [when present]) were dissected and thoroughly washed into sedimentation glasses and examined under a dissection scope for helminths. Additionally, localized tissue damage (e.g., lesions, inflammation) associated with parasite infections was characterized macroscopically and photodocumented.

Nematodes were fixed in acetic acid for 1–5 min and stored in vials of 70% ethyl alcohol plus 8% glycerin solution. They were mounted on microscope slides in alcohol glycerin wet

mounts for identification and counting. Cestodes were fixed in acid-formalin-ethyl alcohol for 20–30 min and then stored in vials of 70% ethyl alcohol. Acanthocephalans were fixed in acid-formalin-ethyl alcohol for 1 h and stored in vials of 70% ethyl alcohol. Cestodes and acanthocephalans were mounted on microscope slides in alcohol wet mounts for identification. If necessary for identification, they were stained with Carmine Alum and mounted in Canada balsam on microscope slides. Voucher specimens were deposited at the Sam Houston State University Parasite Museum, Sam Houston State University, Huntsville, Texas: *Aulonocephalus pennula* (SHSUP000533), *Oxyspirura petrowi* (SHSUP000529), *Tetrameres pattersoni* (SHSUP000530), and *Mediorhynchus papillosus* (SHSUP000535). Parasitologic terminology follows Bush et al. (1997).

Statistical analyses were conducted on individual helminth species with $\geq 25\%$ prevalence across the collective data set to assess the effects of host intrinsic (mass, age, sex) and extrinsic (season of collection) variables on prevalence and abundance. We used SAS 9.3 software (SAS Institute Inc., Cary, North Carolina, USA) for statistical analyses. Chi-square analyses were used to determine if prevalence differed among the main effects variables of host age, host sex, and season of host collection and biologically significant interactions between these variables (host age*host sex and host age*season of host collection). The level of significance was $P \leq 0.05$. Descriptive statistics are presented as a mean \pm SE.

An analysis of variance was used to compare mean masses of infected and noninfected individuals within each age cohort (juvenile and adult). The assumptions of homogenous variances and normality were tested with Levene's test and the Shapiro-Wilk test, respectively.

Helminth abundances were plotted to determine the distribution of the data. A generalized linear mixed model procedure (PROC GLIMMIX) based on this distribution was used to create a model with the use of the main-effects variables to predict helminth abundance. The initial model included the main-effects variables (host age, host sex, host mass, and season of host collection) and interactions of interest (host age*host sex, host age*host mass, and host age*season of host collection). With the use of reverse selection based on the type III effects, the relationship between the number of helminths and the model variables was estimated. Reverse selection demonstrated the independent relationship of each model component to helminth abundance because type III effects provide a level of significance for each individual component after the

TABLE 1. Prevalence (percent of hosts infected), mean intensity (number of individual parasites per infected host), and mean abundance (number of individual parasites per host regardless of infection status) of helminths from 209 whole birds and 35 heads from Northern Bobwhites (*Colinus virginianus*) collected during the 2012–13 and 2013–14 hunting seasons in South Texas, USA. N/A = not applicable.

Helminth species (microhabitats) ^a	Prevalence no. (%)	Intensity		Abundance	
		$\bar{x} \pm SE$	Range	$\bar{x} \pm SE$	Total
<i>Aulonocephalus pennula</i> (C, L)	162 (78)	82.2±7.1	1–585	64.1±6.0	13,404
<i>Oncicola canis</i> (NM)	22 (11)	18.4±5.2	1–103	1.9±0.7	405
<i>Tetrameres pattersoni</i> (P)	21 (10)	2.8±0.7	1–9	0.3±0.1	59
<i>Oxyspirura petrowi</i> (E) ^b	22 (9)	4.9±1.7	1–36	0.4±0.2	107
<i>Mediorhynchus papillosus</i> (SI)	11 (5)	1.4±0.3	1–4	0.1±<0.1	15
<i>Physaloptera</i> sp. (BM)	2 (1)	1.5±0.5	1–2	<0.1±<0.1	3
<i>Skryabinia cesticillus</i> (SI)	1 (1)	2±N/A	2	<0.1±<0.1	2
<i>Fuhrmannetta</i> sp. (SI)	1 (1)	1±N/A	1	<0.1±<0.1	1
Cestode sp. (SI)	1 (1)	1±N/A	1	<0.1±<0.1	1

^a Microhabitats: C = ceca; L = large intestine; NM = neck muscle; P = proventriculus; E = eye surface, nictating membrane, associated glands, and ducts; SI = small intestine; and BM = breast muscle.

^b *Oxyspirura petrowi* calculations based on $n=244$ (whole-body carcasses and detached heads). All other calculations based on $n=209$.

variation due to all of other components of the model has been taken into account.

RESULTS

During the two Texas hunting seasons, 209 whole bobwhites were donated, representing 16 of 31 South Texas counties. The sample of whole quail from the 2012–13 season consisted of 21 adults and 70 juveniles, whereas the 2013–14 sample consisted of 31 adults and 87 juveniles. An additional 35 detached heads were donated during the 2012–13 season.

Helminth fauna

Nine helminth species were found, consisting of four nematode, three cestode, and two acanthocephalan species, which accounted for 13,997 specimens (Table 1). Another cestode species was encountered, but its degenerated condition prevented identification. *Aulonocephalus pennula* was the most prevalent (78%) and abundant species, followed by *Oncicola canis* (11% prevalence). The remaining species each occurred <10% and contributed <1% of total worms found (Table 1). Microhabitats in bobwhites infected with helminths were the ceca, small intestine, large intestine, neck muscles, proventriculus, surface of the

eye, glands and ducts associated with the eye, and breast muscle (Table 1).

Prevalence by host age, sex, and year of collection

Only one species (*A. pennula*) met the criteria for statistical analysis of $\geq 25\%$ prevalence (Table 1). A plot of the abundance data for *A. pennula* indicated a negative binomial distribution with the highest frequencies aggregated toward the lowest counts of worms. *Aulonocephalus pennula* occurred in adults more frequently ($P=0.003$) than juveniles (92% and 73%, respectively). However, no differences ($P = 0.178$) were observed for host sex. Prevalence of *A. pennula* in bobwhites collected during the 2012–13 hunting season (85%) was higher ($P = 0.031$) than those collected during the 2013–14 season (72%). The prevalence of *A. pennula* in adults did not vary ($P=0.514$) by season. Prevalence in juveniles was significantly higher ($P=0.026$) during the 2012–13 hunting season (81%) than the 2013–14 season (66%). No interaction effect was observed ($P=0.586$) for host age and host sex.

Host mass

Mean mass of adults infected with *A. pennula* (163.8 ± 1.8 g) was not significantly different ($P=0.568$) from that of noninfected

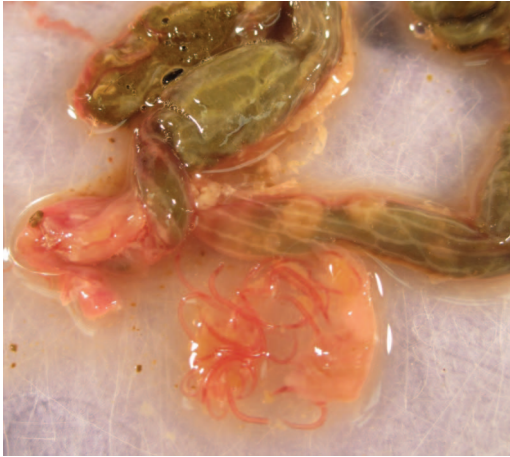


FIGURE 1. *Aulonocephalus pennula* (>300) removed from distended terminal ends of the ceca of a Northern Bobwhite (*Colinus virginianus*) collected in South Texas, USA.

adults (167.5 ± 5.9 g). Mean mass of infected juveniles (158.8 ± 1.1 g) was significantly higher ($P < 0.001$) than that of noninfected juveniles (144.6 ± 3.4 g).

Trend analyses of *A. pennula* abundance

The significant abundance model (abundance=age+mass) indicated that *A. pennula* was more abundant ($P=0.047$) in adults than juveniles and in the heavier quail ($P=0.003$) in each age cohort. The least-squares mean of *A. pennula* abundance in adults was significantly higher than that of juveniles ($P=0.047$).

Gross pathology

With intense *A. pennula* infections (>300 worms), distension of the ceca was observed, particularly at the terminal ends (Fig. 1). Female *T. pattersoni* were observed filling the entire volume of and distending individual glands in the proventriculus (Fig. 2).

DISCUSSION

This research represents the first broad-scale study (16 counties) of bobwhite helminths in South Texas, expanding upon previous studies that despite robust sample sizes, only sampled bobwhites in 2–5 counties

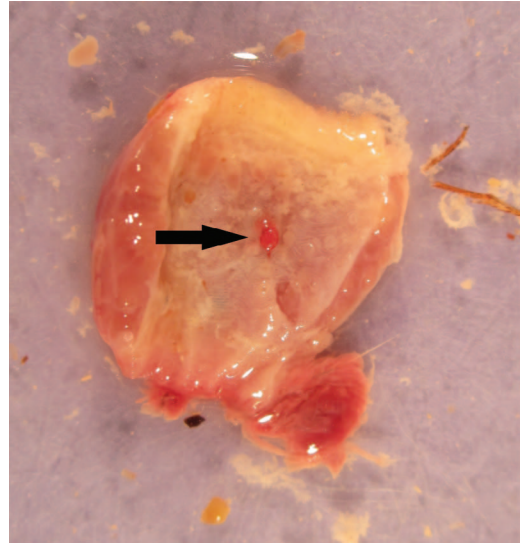


FIGURE 2. A female *Tetrameres pattersoni* partially removed from a gland in the proventriculus and causing localized inflammation in a Northern Bobwhite (*Colinus virginianus*) collected in South Texas, USA.

(Table 2; Webster and Addis 1945; Lehmann 1984; Demarais et al. 1987). We identified more helminth species in our sample of bobwhites than Demarais et al. (1987) and Webster and Addis (1945; Table 2). Lehmann (1984) documented one more helminth species than our study and surveyed fewer counties, but had a much larger sample size (Table 2). Webster and Addis (1945) and Demarais et al. (1987) also collected more bobwhites than we did but our helminth species richness was higher than theirs, reinforcing the importance of broad-scale surveys when describing helminth infections across large regions (Table 2).

Aulonocephalus pennula was the most prevalent and abundant species infecting bobwhites in this study. Two other studies in South Texas reported similar prevalences of *A. pennula* (Webster and Addis, 1945; Lehmann 1984). Demarais et al. (1987) determined *A. pennula* prevalence on two ranches in South Texas for 2 yr and found slightly lower yearly mean prevalences by ranch ranging from 6% to 49%. The reason for the success of *A. pennula* compared to other quail

TABLE 2. Number of counties surveyed, Northern Bobwhites (*Colinus virginianus*) collected, and helminth species documented in four studies conducted in South Texas, USA.

Helminth study	Counties surveyed	Bobwhites collected	Helminth species
Webster and Addis (1945)	4	276	6
Lehmann (1984)	5	718	10
Demarais et al. (1987)	2	481	4
Present study	16	209	9

helminths in South Texas and Texas as a whole is unknown.

The pathologic effects of *A. pennula* infections, if any, are unknown. *Aulonocephalus pennula*, although never observed penetrating the cecal wall, was prevalent and abundant and occasionally occurred at intensities of several hundred worms. The effect of this many *A. pennula* in a single bobwhite warrants further investigation. However, the lack of significant differences in host mass between infected and uninfected adult bobwhites in this study did not provide evidence of deleterious effects of *A. pennula* on the individual health of bobwhites collected during the hunting season.

Here, we document *O. petrowi* infections in bobwhites from South Texas. No macroscopic evidence of pathology associated with *O. petrowi* infections was observed in our study. However, Bruno et al. (2015) presented histologic evidence of tissue damage. Bruno (2014), Dunham et al. (2014), and Villarreal et al. (2012) documented *O. petrowi* in bobwhites from the Rolling Plains with prevalences $\geq 40\%$ (40–97%) across host age, season, and years, which was much higher than the 9% prevalence found. The low prevalence in South Texas compared to the Rolling Plains may result from differences in intermediate host species (presently unknown but likely arthropods) composition and abundance resulting from different habitat types and climates.

Tetrameres pattersoni represented $<1\%$ of total helminths we found in bobwhites. This helminth can be pathogenic to bobwhites. Kellogg and Doster (1972) documented proventriculitis in pen-raised bobwhites infected with *T. pattersoni*. When Cram (1933) initially

described *T. pattersoni* from wild bobwhites collected in North Carolina, she described heavy infections (up to 21 females and seven males in one host) that covered nearly the entire wall of the proventriculus. Villarreal et al. (2012) also reported *T. pattersoni* infections in bobwhites from the Rolling Plains of Texas. Although we did not find *T. pattersoni* at intensities as high as those described by Kellogg and Doster (1972) or Cram (1933), we observed localized inflammation associated with female *T. pattersoni* in the glands of the proventriculus. The pathogenic capabilities of *T. pattersoni* warrant additional research into the prevalence, abundance, and intensity of *T. pattersoni* infections in bobwhites from South Texas and their potential impact on individual hosts and populations.

Given the decline of bobwhites within some areas of South Texas, investigations such as this one into the diseases and parasites of bobwhites and their potential to affect bobwhite health are warranted. Our broad-scale study identified nine helminths infecting bobwhites in South Texas, including two species known to cause tissue damage. The prevalence and abundance documented in this study of another helminth, *A. pennula*, and the lack of understanding of the potential impact of this parasite should motivate continued research on this species.

ACKNOWLEDGMENTS

We thank the San Antonio Livestock Exposition, Inc., Houston Safari Club, Texas Quail Coalition (South Texas Chapter), Title V Promoting Postbaccalaureate Opportunities for Hispanic Americans program, and the Caesar Kleberg Wildlife Research Institute for providing financial support for this research. We are grateful to the

many hunters, ranch managers, hunting guides, and landowners who provided quail for this project. We thank Mike Kinsella for his invaluable expertise on parasite taxonomy and identification and David Wester for his patient assistance with statistical analyses. Two reviewers and an associate editor provided excellent comments and suggestions on a previous version of this manuscript. This is manuscript 16-112 of the Caesar Kleberg Wildlife Research Institute.

LITERATURE CITED

- Brennan LA. 1991. How can we reverse the Northern Bobwhite population decline? *Wildl Soc Bull* 19:544–555.
- Brennan LA. 2014. A legacy landscape for bobwhite conservation. *South Tex Wildl* 18:1–2.
- Brennan LA, Hernández F, Bryant FC. 2007. Introduction. In: *Texas quails: Ecology and management*, Brennan LA, editor. Texas A&M University Press, College Station, Texas, pp. 3–5.
- Bruno A. 2014. *Survey for Trichomonas gallinae and assessment of helminth parasites in northern bobwhites from the Rolling Plains ecoregion*. MS Thesis, Range and Wildlife Science, Texas A&M University–Kingsville, Kingsville, Texas, 133 pp.
- Bruno A, Fedynich AM, Smith-Herron A, Rollins D. 2015. Pathological response of Northern Bobwhites to *Oxyspirura petrowi* infections. *J Parasitol* 101:364–368.
- Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 83:575–583.
- Cram EB. 1933. A new species of *Tetrameres* from the bobwhite. *J Parasitol* 19:245–246.
- Demarais S, Everett DD, Pons ML. 1987. Seasonal comparison of endoparasites of Northern Bobwhites from two types of habitat in southern Texas. *J Wildl Dis* 23:256–260.
- Dunham NR, Soliz LA, Fedynich AM, Rollins D, Kendall RJ. 2014. Evidence of an *Oxyspirura petrowi* epizootic in Northern Bobwhite (*Colinus virginianus*), Texas, USA. *J Wildl Dis* 50:552–558.
- Fulbright TE, Bryant FC. 2003. The Wild Horse Desert: Climate and ecology. In: *Ranch management: Integrating cattle, wildlife, and range*, Forgason CA, Bryant FC, Genho PC, editors. King Ranch Institute, Kingsville, Texas, pp. 35–58.
- Hernández F, Guthery FS. 2012. General ecology. In: *Beef, brush, and bobwhites: Quail management in cattle country*, Hernández F, Guthery FS, editors. Texas A&M University Press, College Station, Texas, pp. 15–31.
- Hernández F, Guthery FS, Kuvlesky WP Jr. 2002. The legacy of bobwhite research in South Texas. *J Wildl Manage* 66:1–18.
- Hernández F, Peterson MJ. 2007. Northern Bobwhite ecology and life history. In: *Texas quails: Ecology and management*, Brennan LA, editor. Texas A&M University Press, College Station, Texas, pp. 40–64.
- Ingles JM. 1964. *A history of vegetation on the Rio Grande Plain*. Project W-84-R-Texas, Bulletin No. 45. Texas Parks and Wildlife Department, Austin, Texas, 122 pp.
- Kellogg FE, Doster GL. 1972. Diseases and parasites of the bobwhite. In: *Proceedings of the first national bobwhite quail symposium*, Morrison JA, Lewis JC, editors. Stillwater, Oklahoma, 23–26 April, pp. 233–267.
- Lehmann VW. 1946. Bobwhite quail reproduction in southwestern Texas. *J Wildl Manage* 10:111–123.
- Lehmann VW. 1984. *Bobwhites in the Rio Grande Plain of Texas*. Texas A&M University Press, College Station, Texas, 371 pp.
- Leopold AS. 1939. Age determination in quail. *J Wildl Manage* 3:261–265.
- Peterson MJ. 2007. Diseases and parasites of Texas quails. In: *Texas quails: Ecology and management*, Brennan LA, editor. Texas A&M University Press, College Station, Texas, pp. 89–114.
- Silvy NJ, Rollins D, Whisneman SW. 2007. Scaled quail ecology and life history. In: *Texas quails: Ecology and management*, Brennan LA, editor. Texas A&M University Press, College Station, Texas, pp. 65–88.
- Villarreal SM, Fedynich AM, Brennan LA, Rollins D. 2012. Parasitic eyeworm *Oxyspirura petrowi* in Northern Bobwhites from the Rolling Plains of Texas, 2007–2011. In: *Proceedings of the 7th national quail symposium*, National Bobwhite Conservation Initiative, Tucson, Arizona, 9–12 January, pp. 241–243.
- Webster JD, Addis CJ. 1945. Helminths from the bobwhite quail in Texas. *J Parasitol* 31:286–287.

Submitted for publication 20 November 2015.

Accepted 11 February 2016.