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## HELMINTH INFECTIONS ACROSS A NORTHERN BOBWHITE (*COLINUS VIRGINIANUS*) ANNUAL CYCLE IN FISHER COUNTY, TEXAS

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**ABSTRACT.**—Northern Bobwhite (*Colinus virginianus*) populations in Texas have been declining during the past several decades. Declines have been attributed to habitat loss, but other causes and potential contributing factors (e.g., parasites, disease) have been posited. Little is known about helminth parasites in bobwhites from Texas. Previous studies often used bobwhites collected during the hunting season, which only samples individuals that survive after the summer breeding season. Our objectives were to (1) assess the prevalence, intensity, and abundance of helminths in bobwhites from Fisher County, Texas, during an annual cycle; (2) identify which species are known to be pathogenic; and (3) determine whether infections are related to host age, sex, and season of collection. We collected 142 bobwhites during February–March 2010 ( $n = 37$ ), August 2010 ( $n = 51$ ), and December 2010–January 2011 ( $n = 54$ ). We found 7 helminth species, of which 3 (*Oxyspirura petrowi*, *Tetrameres pattersoni*, and *Dispharynx nasuta*) are known to cause tissue damage to bobwhites. *Aulonocephalus pennula* was the most common (82% prevalence) and numerically abundant species (96% of all specimens). Prevalence and mean abundance of *A. pennula*, *O. petrowi*, and *T. pattersoni* were higher in adults than juveniles. Prevalence of *A. pennula*, *O. petrowi*, and *T. pattersoni* did not vary with host sex. Mean abundance of *T. pattersoni* was higher in females than males. Prevalence of *A. pennula*, *O. petrowi*, and *T. pattersoni* was lower in summer than early and late winter and was related to low infections in young juveniles. Our findings provide insight into helminth infection dynamics of Northern Bobwhites across an annual cycle.

**RESUMEN.**—Las poblaciones de codorniz nortea (*Colinus virginianus*) en Texas han disminuido durante las últimas décadas. En general, las disminuciones se han atribuido a la pérdida del hábitat, pero también se han planteado otros factores que podrían contribuir (como parásitos, enfermedades, etc.). Sabemos poco sobre los parásitos helmintos en las codornices de Texas. A menudo, estudios previos han utilizado codornices colectadas durante la temporada de caza, lo que muestrea sólo aquellos individuos que sobrevivieron la temporada de reproducción del verano. Nuestros objetivos fueron (1) evaluar la prevalencia, intensidad y abundancia de helmintos en codornices en el condado de Fisher, Texas, durante un ciclo anual, (2) identificar qué especies se sabe que son patógenas, y (3) determinar si las infecciones están relacionadas con la edad, el sexo del hospedero y con la temporada de colecta. Colectamos 142 codornices durante febrero–marzo del 2010 ( $n = 37$ ), agosto del 2010 ( $n = 51$ ) y diciembre del 2010 a enero del 2011 ( $n = 54$ ). Encontramos 7 especies de helmintos de las cuales 3 (*Oxyspirura petrowi*, *Tetrameres pattersoni* y *Dispharynx nasuta*) son conocidas por causar daños en los tejidos de las codornices. *Aulonocephalus pennula* fue la más común (un 82% de prevalencia) y abundante (96% de todas las muestras) de las especies. La prevalencia y la abundancia media de *A. pennula*, *O. petrowi* y *T. pattersoni* fueron mayores en los adultos que en los juveniles. La prevalencia de *A. pennula*, *O. petrowi* y *T. pattersoni* no varió con el sexo del hospedero. La abundancia promedio de *T. pattersoni* fue mayor en las hembras que en los machos. La prevalencia de *A. pennula*, *O. petrowi* y *T. pattersoni* fue menor en verano que en invierno y se relacionó con infecciones leves en ejemplares jóvenes. Nuestros resultados proporcionan una idea sobre la dinámica de las infecciones por helmintos en las codornices del norte a lo largo del ciclo anual.

The Northern Bobwhite (*Colinus virginianus*; hereafter, bobwhite) is a popular game bird in Texas, but its populations have been declining for more than 4 decades. These population declines have been attributed to habitat loss (Hernández et al. 2013). However, Peterson (2007) commented that parasites may play a role in population declines and suggested more information was needed on this topic.

One of the few regions where bobwhites have maintained abundant populations is the Rolling Plains of Texas (Rollins 2007). However, our knowledge of the parasite community in this region is limited to a study that included an unknown number of bobwhites from Coleman County in a sample of 14 individuals from central Texas (Webster and Addis 1945); a study that examined 605 bobwhites for eyeworms, 229 for eye and breast muscle

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nematodes, and 47 for eye, breast, and intestinal nematodes (Jackson and Green 1965); and a study of 151 bobwhites (Jackson 1969) in which completeness of necropsy methods was unclear. In addition, several recent studies have focused exclusively on eyeworms (Dunham et al. 2014, Bruno et al. 2015), which does not provide insight about the helminth community. Consequently, it is unknown what helminth species currently infect bobwhites within the Rolling Plains and whether there are pathogenic species present.

Evidence from studies conducted in northern Florida suggest that host age influences parasite infections of bobwhites (Moore et al. 1987, Davidson et al. 1991). Historically, studies have used samples collected only during the hunting season which results in a sample of individual quail that have already survived various parasite challenges. Therefore, it is necessary to collect young juveniles (6–10 weeks old), older juveniles (>10 weeks old), and adults to examine host age within the context of seasonal infections. Our objectives were to determine species of helminth parasites infecting bobwhites in Fisher County, Texas; identify species known to be pathogenic; and determine whether prevalence and abundance of helminths vary with host age, host sex, and season.

#### METHODS

We collected 142 bobwhites (45 adult males, 23 adult females, 37 juvenile males, and 37 juvenile females) in Fisher County, Texas, at the Rolling Plains Quail Research Ranch (RPQRR) (32°43'14.65"N, 100°32'49.89"W) and a nearby private ranch located 22.5 km east of the RPQRR (32°43'9.01"N, 100°19'15.01"W). The RPQRR is a nonprofit research ranch. These ranches are approximately 1902 ha and 1416 ha in size, respectively.

There were 3 collection periods: late winter (February–March 2010; 25 adults and 12 old juveniles >10 weeks old), summer (August 2010; 22 adults and 29 young juveniles 6–10 weeks old), and early winter (December 2010–January 2011; 21 adults and 33 old juveniles >10 weeks old). These time intervals were selected so that the critical periods (i.e., birds surviving the winter and entering the breeding season, summer breeding season, and birds entering the winter season) of the annual

host cycle could be assessed for helminths. We did not collect during the nesting season (April–July).

We collected bobwhites by shooting during the hunting season (opportunistically using hunter-shot birds) and by shooting under the auspices of a scientific collection permit outside of the hunting season (Texas Parks and Wildlife Department; permit SPR-1209-400). We determined the sex of each host by using the coloration of feathers on the head. Age of juveniles and adults was determined by examining the primary wing coverts (Jackson 1969). We classified flight-capable juveniles as young juveniles if they were collected in August (6–10 weeks old) and as old juveniles if they were collected during February–March or December–January (>10 weeks old). Each bird was transported from the field in an individually numbered plastic freezer bag on ice and later frozen. Birds were necropsied, and parasites were processed and identified as outlined in Villarreal (2012). Since most of the cestode samples were deteriorated and scolices were often missing, species could not be readily identified or direct counts made. Therefore, multiple cestode fragments found in which genus could be determined were counted as one cestode individual per bird. Representative specimens were deposited in the U.S. National Parasite Collection, Beltsville, MD: *Aulonocephalus pennula* (105783), *Oxyspirura petrowi* (105784), *Tetrameres patersoni* (105785), and *Dispharynx nasuta* (105786). Parasitological terms of prevalence, intensity of infection, abundance, mean abundance, infracommunity, and component community follow Bush et al. (1997).

We used chi-square analysis to compare the prevalence of parasite species that occurred  $\geq 25\%$  for the main effects variables of host age, sex, and season of collection. To account for the aggregated count data, we used a generalized linear mixed model fitted with the negative binomial distribution (PROC GLIMMIX) in SAS 9.3 software (SAS Institute Inc., Cary, NC). We sought to determine whether abundance of the parasites varied by the main (age, sex, and season) and relevant interaction effects variables (age \* sex and age \* season). We used backward selection based on type III effects  $F$  tests to eliminate terms where  $P > 0.05$  until only significant effects remained. The type III effects test the significance of an

TABLE 1. Prevalence values (number of infected and percentage infected), intensity values (mean [SE]), range, abundance (mean [SE]), and total for helminths from 142 Northern Bobwhites (*Colinus virginianus*) collected from Fisher County, Texas, during 2010–2011.

Helminth species	Prevalence	Intensity		Abundance	
	No. (%)	Mean (SE)	Range	Mean (SE)	Total
<i>Aulonocephalus pennula</i> (C, SI, L) <sup>a</sup>	117 (82)	134.3 (11.2)	2–518	110.7 (10.2)	15,716
<i>Oxyspirura petrowi</i> (E)	67 (47)	5.6 (0.7)	1–23	2.6 (0.4)	373
<i>Tetrameres pattersoni</i> (P)	37 (26)	2.9 (0.3)	1–8	0.8 (0.1)	108
<i>Dispharynx nasuta</i> (P)	1 (0.7)	1	1	<0.1 (<0.1)	1
Acanthocephalan larvae (N)	26 (18)	4.3 (1.7)	1–43	0.8 (0.3)	113
Cestode spp. <sup>b</sup> (SI)	13 (9)	—	—	—	13

<sup>a</sup>Microhabitats: C = ceca, E = eye, L = large intestine, N = neck muscle, P = proventriculus, SI = small intestine.

<sup>b</sup>*Rhabdometra odiosa* and *Raillietina* spp. were combined due to poor quality specimens and were counted as present or absent and one individual per infected host.

effect when all other effects are included in the specified model. We used least-squares means to compare significant effects among variables. Statistical significance was inferred at  $P \leq 0.05$ . Descriptive statistics are presented as the mean  $\pm$  1 standard error (SE).

## RESULTS

We found 7 species of helminths (4 nematodes, 1 acanthocephalan larva, and 2 cestodes) representing 16,324 helminth individuals (Table 1). We found helminths in 6 microhabitats: under the nictitating membrane of the eye and in neck muscle, proventriculus, small intestine, large intestine, and ceca (Table 1).

Overall, 15% of the bobwhites were not infected, 28% were infected with 1 species, 30% with 2 species, 21% with 3 species, 5% with 4 species, and 1% with 5 species. Nematodes were the most abundant taxon (Table 1). The most prevalent and numerically abundant species was *A. pennula* (82% prevalence and 96% of all worm individuals), followed by *O. petrowi* (47% and 2%) and *T. pattersoni* (26% and 0.7%). All of the remaining species had <19% prevalence and contributed <1% of worms (Table 1). Cestodes (*Rhabdometra odiosa* and *Raillietina* spp.) occurred in 13 (9%) birds (Table 1).

### Influence of Host Age

Prevalence of *A. pennula* in adults and old juveniles was greater ( $P < 0.0001$ ) than in young juveniles (97%, 100%, and 21%, respectively), whereas prevalence of *O. petrowi* and *T. pattersoni* were greater ( $P < 0.0001$  for each species) in adults (68% and 43%, respectively) than in old juveniles (42% and 18%, respectively) and young juveniles (7% and 0%,

respectively; Table 2). An interaction between host age and sex was found for mean abundance of *A. pennula* ( $P = 0.0004$ ). Mean abundance of *A. pennula* was similar between adult males and females and old juvenile males and females but was lower in young juvenile males than females ( $P < 0.0001$ ). Mean abundance of *O. petrowi* was similar between adults and old juveniles but was lower ( $P < 0.0001$ ) in young juveniles. Mean abundance of *T. pattersoni* was higher ( $P < 0.0001$ ) in adults than old juveniles; *T. pattersoni* was not found in young juveniles.

### Influence of Host Sex

Prevalence of *A. pennula*, *O. petrowi*, and *T. pattersoni* did not vary by host sex. We found no differences between host sex and mean abundance of *O. petrowi*. Mean abundance of *T. pattersoni* was higher ( $P = 0.0051$ ) in females compared to males. The influence of host sex on *A. pennula* was significant only within young juveniles as explained above.

### Influence of Season

All bobwhites were infected with at least one parasite species in the late-winter collection; 59% were infected with 1–2 species and 41% were infected with 3–4 species. In summer, 33% were infected with 1–2 species and 24% were infected with 3–4 species, but these numbers were influenced by lack of infections in young juveniles (Table 3). In the early winter collection, 80% were infected with 1–2 species and 20% were infected with 3–5 species.

Variation in prevalence (late winter, summer, early winter) occurred for *A. pennula* ( $P < 0.0001$ ; 100%, 55%, and 96%, respectively), *O. petrowi* ( $P = 0.0137$ ; 65%, 33%, 48%), and *T. pattersoni* ( $P = 0.0215$ ; 43%, 20%, 20%).

TABLE 2. Prevalence (number of infected and % infected), abundance (mean [SE]), and total for helminths from 142 Northern Bobwhites (*Colinus virginianus*) by host age from Fisher County, Texas, during 2010–2011.

Helminth species	Adult (n = 68)			Old juvenile <sup>a</sup> (n = 45)			Young juvenile <sup>b</sup> (n = 29)		
	Prevalence		Abundance	Prevalence		Abundance	Prevalence		Abundance
	No. (%)	Mean (SE)	Total	No. (%)	Mean (SE)	Total	No. (%)	Mean (SE)	Total
<i>Aulonocephalus pennula</i>	66 (97)	146.1 (14.3)	9937	45 (100)	124.3 (19.1)	5592	6 (21)	6.4 (5.0)	187
<i>Oxyurisura petrowi</i>	46 (68)	3.9 (0.6)	262	19 (42)	2.4 (0.7)	108	2 (7)	0.1 (0.1)	3
<i>Tetrameres pattersoni</i>	29 (43)	1.4 (0.3)	93	8 (18)	0.3 (0.1)	15	0 (0)	—	—
<i>Dispharynx nasuta</i>	1 (1)	<0.1 (<0.1)	1	0 (0)	—	0	0 (0)	—	—
Acanthocephalan larvae	22 (32)	1.6 (0.7)	109	4 (9)	0.1 (<0.1)	4	0 (0)	—	—
Cestode spp. <sup>c</sup>	10 (15)	—	10	1 (2)	—	1	2 (7)	—	2

<sup>a</sup>Old juveniles are those >10 weeks old.

<sup>b</sup>Young juveniles are those 6–10 weeks old.

<sup>c</sup>*Blabidometra odiosa* and *Rallitina* spp. were combined due to poor quality specimens and were counted as present or absent and one individual per infected host.

TABLE 3. Prevalence (number of infected and % infected), abundance (mean [SE]), and total for the 3 common (≥25% prevalence) helminth species in 74 juvenile and 68 adult Northern Bobwhites (*Colinus virginianus*) by season from Fisher County, Texas, during 2010–2011.

Species	Late winter 2010			Summer 2010			Early winter 2010–2011					
	Juveniles (n = 12) <sup>a</sup>		Adults (n = 25)	Juveniles (n = 29) <sup>b</sup>		Adults (n = 22)	Juveniles (n = 33) <sup>a</sup>		Adults (n = 21)			
	Prevalence No. (%)	Abundance mean (SE)	Abundance	Prevalence No. (%)	Abundance mean (SE)	Abundance	Prevalence No. (%)	Abundance mean (SE)	Abundance			
<i>Aulonocephalus pennula</i>	12 (100)	204.1 (44.2)	25 (100)	175.0 (22.2)	6 (21)	6.4 (5.0)	22 (100)	129.9 (20.7)	33 (100)	95.2 (18.5)	19 (90)	128.7 (31.1)
<i>Oxyurisura petrowi</i>	5 (42)	1.1 (0.5)	19 (76)	5.2 (1.2)	2 (1)	0.1 (0.1)	15 (68)	2.7 (0.7)	14 (42)	2.9 (0.9)	12 (57)	3.5 (1.3)
<i>Tetrameres pattersoni</i>	3 (25)	0.3 (0.1)	13 (52)	1.7 (0.5)	0 (0)	—	10 (45)	1.3 (0.4)	5 (15)	0.4 (0.2)	6 (29)	1.0 (0.5)

<sup>a</sup>Old juveniles are those >10 weeks old.

<sup>b</sup>Young juveniles are those 6–10 weeks old.

However, mean abundance of *A. pennula*, *O. petrowi*, and *T. pattersoni* did not vary by season.

#### DISCUSSION

We found depauperate helminth infra-communities, ranging from 1 to 5 species, in which the most common was 2 helminth species (30%) within an individual. The component community consisted of 7 species, all with indirect life cycles, and was dominated by *A. pennula*. In contrast, studies conducted in the eastern and southeastern United States found wild bobwhites to have species-rich ( $\geq 19$  species) helminth communities including species with direct and indirect life cycles (Cram et al. 1931, Davidson et al. 1982, Forrester and Spalding 2003). The reason for these different findings is unclear, but a likely factor is different precipitation regimes between geographic regions, where drier conditions may negatively influence intermediate host population dynamics and limit parasites with direct life cycles (Gray et al. 1978).

*Oxyuris petrowi* and *T. pattersoni* occurred in  $\geq 25\%$  prevalence. Localized tissue damage and inflammation have been reported for *T. pattersoni* (Davidson et al. 1991) and *O. petrowi* (Bruno et al. 2015). We probably undercounted *O. petrowi* by examining only the eye surface and underneath the eyelid and nictitating membrane because recent reports indicate that *O. petrowi* also occurs in the lacrimal duct, lacrimal gland, and Harderian gland (Dunham et al. 2014, Bruno et al. 2015).

We report for the first time *T. pattersoni* and *D. nasuta* in Northern Bobwhites from the Rolling Plains ecoregion of Texas. *Tetrameres pattersoni* is thought to be more pathogenic than *Tetrameres americana* (Peterson 2007), and *D. nasuta* is considered the most pathogenic of the 15 species of *Dispharynx* (Carreno 2008). Unfortunately, studies have not been conducted to determine whether *T. pattersoni* and *D. nasuta* have an impact at the host population level.

#### Influence of Host Age

Young juvenile bobwhites had few or no helminth species, whereas old juveniles and adults had helminth communities that were similar in species richness. Prevalence and abundance of the 3 common species tended to be related to host age. Some bobwhite parasite

studies have shown host age to influence some species of helminths (Davidson et al. 1991), whereas other studies have not (Forrester et al. 1984, Moore et al. 1987). Parmalee (1952) found no difference between host age and parasitism in bobwhites from Texas. Based on our findings, bobwhites are accumulating both species and individuals of species as they transition from hatch-year birds to adults.

#### Influence of Host Sex

Host sex was not an important factor in prevalence or abundance of *O. petrowi*, indicating that both sexes are similarly exposed to infective stages of this helminth. However, mean abundance of *A. pennula* was higher in young juvenile females than in the other age-sex categories, and mean abundance of *T. pattersoni* was higher in females than males. Our results are generally consistent with the findings of other studies in which host sex has little or no influence on helminth prevalence or intensity of infection (Forrester et al. 1984, Demarais et al. 1987, Moore et al. 1987, Davidson et al. 1991). Of the 15 species found in bobwhites from Florida by Davidson et al. (1991), 2 nematodes (*Cyrtus colini* and *Heterakis isolonche*) were higher in females than males.

#### Influence of Season

Several studies report a seasonal effect in particular species of helminths, in which prevalence, intensity, or abundance vary (Hansen and Robel 1972, Davidson et al. 1980, Demarais et al. 1987). Davidson et al. (1980) demonstrated that certain species tend to increase with time, whereas others remain relatively stable or fluctuate erratically. In our study, fewer bobwhites were infected with *A. pennula*, *O. petrowi*, and *T. pattersoni* in the summer than in late winter or early winter. This pattern was strongly influenced by young juveniles, which had few to no helminths.

In a methodological change from previous studies, we separated juveniles into 2 categories (6–10 weeks old and >10 weeks old). We believe this classification clarified the infection pattern wherein young juveniles started to become infected (69% did not acquire infections and those that did had 1 or 2 species), compared to old juveniles where helminth community pattern and structure were more similar to that found in adults. This finding underscores the importance of subclassifying

juveniles for a better understanding of how helminth communities develop in specific host age classes and also demonstrates that sampling during the winter hunting season likely provides the best snapshot of overall helminth community composition in bobwhites.

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