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GEOGRAPHIC SURVEY OF *OXYSPIRURA PETROWI* AMONG WILD NORTHERN BOBWHITES IN THE UNITED STATES

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ABSTRACT

Eyeworms (*Oxyspirura petrowi*) are potentially associated with northern bobwhite (*Colinus virginianus*) declines. We examined hunter-donated bobwhites from the 2013–2015 hunting seasons in 9 states to document infection prevalence (% of bobwhites [of total n]) and intensity (mean no. of eyeworms ± SE). Four states harbored infected bobwhites: Texas (59.1% [$n = 110$], 15.6 ± 2.1), Oklahoma (52.1% [$n = 121$], 6.9 ± 1.2), Virginia (14.8% [$n = 27$], 2.5 ± 1.0), and Alabama (1.6% [$n = 61$], 2.0). Prevalence and intensity of eyeworms in the Texas Rolling Plains were greater ($P < 0.001$ and $P = 0.002$, respectively) than in any other area sampled. Based on our survey, eyeworms are locally prevalent and abundant in bobwhites from the Rolling Plains ecoregion, but virtually nonexistent in many areas that we surveyed.

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Key words: *Colinus virginianus,* eyeworm, northern bobwhite, *Oxyspirura petrowi,* Rolling Plains

The northern bobwhite (*Colinus virginianus*; hereafter, bobwhite) has experienced declining populations across its geographic range for ≥40 years with the ultimate cause of the decline attributed to habitat loss and fragmentation (Hernández and Guthery 2012, Sauer et al. 2014). However, bobwhites are also declining in areas where ample habitat remains (e.g., parts of TX and OK) suggesting that other factors may be involved in the quail decline (Dunham et al. 2014). Recently, researchers in Texas began to revisit parasitic infections in bobwhites to 1) update survey records from the late 1960s (Jackson and Green 1965, Jackson 1969), and 2) investigate parasitic infections as a potential factor in bobwhite population declines. A survey completed by Villarreal et al. (2016) in 2012 in Fisher County, Texas, found the eyeworm, *Oxyspirura petrowi,* to be common (57%) in bobwhites. This prompted further surveys to elucidate the geographic range of *O. petrowi* in wild bobwhites, particularly in areas where bobwhite populations have experienced significant decline.

Eyeworms are heteroxenous, indirect life-cycle nematodes that parasitize the orbital cavity, intraorbital glands, and nasal sinuses of ≥28 avian species in North America (Pence 1972, Dunham et al. 2014, Bruno et al. 2015; Fig. 1). The intermediate host for *O. petrowi* is unknown; however, Surinam cockroaches (*Pycnoscalus surinamensis*) are known intermediate hosts for *O. mansoni,* a similar eyeworm found in domestic chickens (Schwabe 1951). Kistler et al. (2016) successfully infected bobwhites with third-stage larvae via Plains lubber grasshoppers (*Brachystola magna*). However, lubbers are not a known food source for bobwhites. Thus, the intermediate host is still considered an unknown arthropod. Infected bobwhites may exhibit keratitis (i.e., scarring of the cornea) and other signs associated with inflammatory responses (Bruno et al. 2015). Histological results do not imply whether infection causes visual impairment or reduced fitness, but coupled with high prevalence, *O. petrowi* infections warrant further investigation.

After the initial survey by Villarreal et al. (2016; 2009–10), recent surveys from the Rolling Plains of Texas and Oklahoma documented *O. petrowi* prevalence ranging from 50% to 100% in bobwhites (Dunham et al. 2014, 2016; Villarreal et al. 2016). High prevalence in this region is consistent with results from surveys by Jackson and Green (1965) finding *O. petrowi* at 44% prevalence ($n = 605$). Outside of Texas, *O. petrowi* has been recorded in bobwhites from Louisiana (Palermo and Doster 1970) and Florida (Davidson et al. 1991), but at very low prevalence (<1%). These earlier accounts in the Southeast dismissed eyeworm infections as extremely rare and probably

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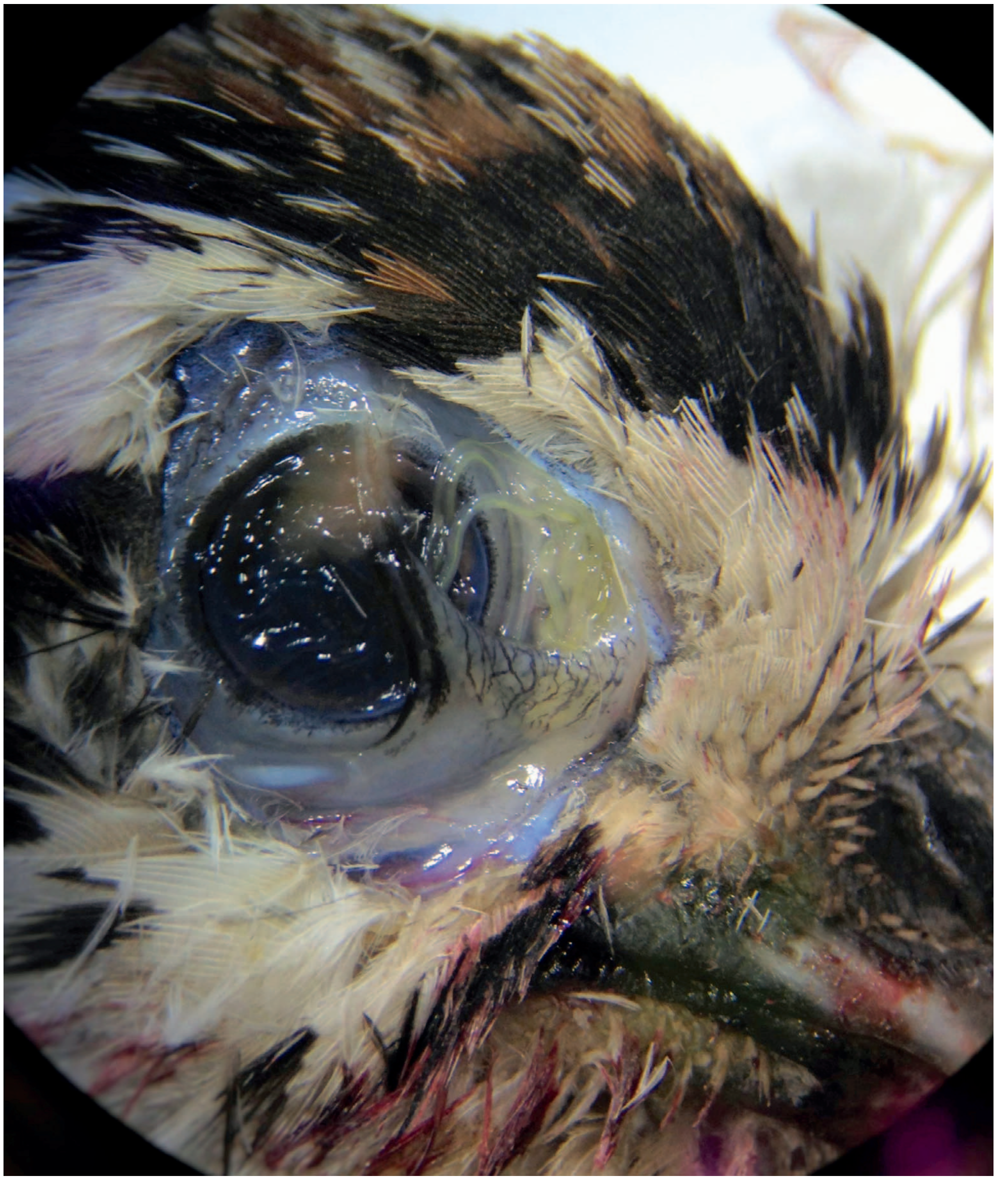

Fig. 1. Orbital cavity of a northern bobwhite (*Colinus virginianus*) infected with *Oxyspirura petrowi*. Nictitating membrane has been removed to reveal several eyeworms aggregated at the right lacrimal.

incidental in wild bobwhites (Kellogg and Calpin 1971, Kellogg and Doster 1972, Davidson et al. 1982). It is likely many earlier studies overlooked or dismissed the importance and presence of parasites in intraorbital glands (Peterson 2007, Dunham et al. 2014). Therefore, our objective was to thoroughly examine and opportunistically survey wild bobwhites from across the United States to determine geographic prevalence and intensity.

STUDY AREA

Heads of wild bobwhite and respective wing samples were collected via hunter-shot donations and miscellaneous submissions to the Rolling Plains Quail Research Ranch from agency biologists in 9 states and from hunters and landowners in Texas during January 2013–February 2016. Our sample ($n = 782$) consisted of birds submitted from Alabama, Georgia, Iowa, Kentucky, Louisiana, Missouri, Oklahoma, Texas, and Virginia.

METHODS

We instructed hunters to freeze all samples as soon as possible after death to prevent deterioration and possible emigration of the eyeworms. We thawed frozen samples in a refrigerator overnight before examination. Using curved forceps and dissecting scissors, we removed the outer eyelids before examining beneath the nictitating membrane. Although eyeworms are apparent to the naked eye, we used a stereo zoom microscope (7×–45×) and a 3 diopter (1.75×) magnifying lens with light-emitting-diode illumination to assure detection. After examining and removing any eyeworms residing beneath the nictitating membrane, we removed the eyes and separated the

Table 1. Prevalence (percent of hosts infected), mean intensity (average eyeworms per infected host), and mean abundance (average for total sample) of *Oxyspirura petrowi* from northern bobwhites (*Colinus virginianus*) sampled from wild, hunter-donated bobwhites harvested during the 2013–2015 hunting seasons across 9 states in the United states.

State	*n*	Prevalence no. (%)	Intensity $\bar{x} \pm$ SE	Intensity Range	Abundance $\bar{x} \pm$ SE	Abundance Total
AL	61	1 (2)	2 ± N/A	1–2	<0.1 ± <0.1	2
GA	79	0	0	0	0	0
IA	56	0	0	0	0	0
KY	36	0	0	0	0	0
LA	25	0	0	0	0	0
MO	267	0	0	0	0	0
OK	121	63 (52)	6.9 ± 1.2	1–56	3.6 ± 0.7	433
VA	27	4 (15)	2.5 ± 1.0	1–5	0.4 ± 0.2	10
TX[a]	110	65 (59)	15.6 ± 2.1	1–79	9.2 ± 1.4	1,015

[a] Rolling Plains ecoregion (Gould 1975).

Harderian and lacrimal glands. We then dissected and examined the nasal sinuses. We fixed all eyeworms recovered for 10 minutes in glacial acetic acid before preserving them in a solution of 70% ethyl alcohol and 8% glycerol. Definitive identification was accomplished examining morphological characteristics described by Pence (1972) under a Leica EZ4D dissection microscope (Leica Microsystems, Wetzlar, Germany). We documented age (e.g., juvenile, adult) and sex of bobwhites.

We conducted Chi-square analysis using PROC FREQ in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) to compare eyeworm prevalence between bobwhite age and sex classes by state. We tested for normality of eyeworm intensity using PROC UNIVARIATE. Data of eyeworm abundance and intensity were not normally distributed. Thus, we used PROC NPAR1WAY to compare mean intensity and mean abundance for bobwhite age and sex classes by state, determining significance at $P \leq 0.05$. Means are expressed as mean ± standard error (SE).

In an effort to standardize terminology, parasitological definitions presented herein follow Bush et al. (1997) where "prevalence" describes percent of infected individuals in a sample; "mean abundance" describes average number of eyeworms among all samples (i.e., infected and noninfected), and "average intensity" describes the average number of eyeworms within the subset of infected individuals only.

RESULTS

We examined the eyes, intraorbital glands, and sinuses of 782 wild bobwhite from 9 states: Alabama ($n = 61$), Georgia ($n = 79$), Iowa ($n = 56$), Kentucky ($n = 36$), Louisiana ($n = 25$), Missouri ($n = 267$), Oklahoma ($n = 121$), Virginia ($n = 27$), and Texas ($n = 110$; Table 1). In Texas, wings were not submitted with every head sample so not all bobwhite ages could be recorded ($n = 26$).

Four of the 9 states had bobwhites that hosted *O. petrowi*—Alabama, Oklahoma, Texas, and Virginia. Prevalence varied greatly among the 4 states from which eyeworms were identified. Only 1 adult male bobwhite from Alabama was infected with 2 eyeworms (1.6% prevalence) while Texas, Oklahoma, and Virginia had 59.1% ($n = 65$), 52.1% ($n = 63$), and 14.8% ($n = 4$) prevalence, respectively (Table 1). Average intensities were 15.6 ± 2.1 (95% CI = 13.5–17.7), 6.9 ± 1.2 (95% CI = 5.7–8.1), and 2.5 ± 1.0 for Texas, Oklahoma, and Virginia, respectively. Texas had a greater mean abundance (9.2 ± 1.4) of eyeworms than did Alabama (0.1 < 0.1), Oklahoma (3.6 ± 0.7), or Virginia (0.4 ± 0.2; $F = 24.6$, $P < 0.001$; Table 1).

Prevalence was similar between sexes for bobwhites from Oklahoma (Yates $\chi^2 = 0.48$, $P = 0.49$) and Texas (Yates $\chi^2 = 0.19$, $P = 0.68$). Pooling across Texas and Oklahoma, prevalence was also similar between sexes (Yates $\chi^2 = 0.83$, $P = 0.36$). Prevalence was similar between juvenile and adult bobwhites in Texas (Yates $\chi^2 = 2.13$, $P = 0.144$).

Mean intensity was similar between males and females in Texas ($P = 0.41$) and Oklahoma ($P = 0.43$). Pooled across Oklahoma and Texas, mean intensities were also similar ($P = 0.16$). Mean intensity between juveniles and adult bobwhites in Texas approached significance ($P = 0.06$) with adults ($n = 12$) having greater intensities (18.8 ± 5.1) than did juveniles (9.4 ± 2.3; $n = 27$).

DISCUSSION

The bobwhites from Texas and Oklahoma sampled in this study came from the Rolling Plains ecoregion located in the northwestern part of Texas extending into western Oklahoma (Gould 1975). Previous studies from areas surrounding this region have reported *O. petrowi* prevalence among bobwhites and other Galliformes ranging from 3% to 95% (Pence and Sell 1979; Pence et al. 1980, 1983; Robel et al. 2003). Eyeworms appear to be enzootic and prolific in this ecoregion since at least the early 1960s (Jackson and Green 1965, Jackson 1969). By contrast, bobwhites in the Rio Grande Plains of southern Texas are less parasitized by eyeworms. Olsen and Fedynich (2016) examined 244 bobwhites during 2012–2014 in the South Texas Plains ecoregion and reported considerably lower *O. petrowi* prevalence (9%) and intensity (4.9 ± 1.7) compared with our estimates from the Rolling Plains. This geographic difference within Texas warrants further investigation. Speculation of higher localized prevalence and intensities in the Rolling Plains could be attributed to intermediate host distribution and abundance, transmission rates (i.e., proportion of individuals that become infected from exposure to agent), or other undetermined factors. Evidence suggests that density-dependent, intermediate-host dynamics exists where parasite abundance is assumedly greater because of greater arthropod abundance and diversity, but this occurrence has not been quantified (Landgrebe et al. 2007).

Although our study did not find any significant differences by age, previous studies on bobwhites from Texas noted significantly higher prevalence and abundance of *O. petrowi* in adult bobwhites (Jackson and Green 1965; Dunham et al. 2014, 2016; Villarreal et al. 2016). Jackson and Green (1965) were the first to note eyeworm infections in wild bobwhites in the Rolling Plains of Texas with prevalence of 44% and intensity ranging from 1 to 30. Our data suggest similar prevalence and intensity to those reported by Jackson and Green (1965) and other recent accounts in the Rolling Plains (Dunham et al. 2014, 2016; Villarreal et al. 2016).

Oxyspirura petrowi has been reported previously in other Galliformes in states outside of Texas and Oklahoma. *Oxyspirura petrowi* was reported in <1% ($n = 203$) of ruffed grouse (*Bonasa umbellus*) in Minnesota (Erickson et al. 1949), 32% ($n = 149$) of sharp-tailed grouse (*Tympanuchus phasianellus*) and in greater prairie-chickens (*T. cupido;* Saunders 1935, Cram 1937) from Michigan. More notable infections of *O. petrowi* were found in 47% ($n = 57$) of ring-necked pheasants (*Phasianus colchicus*) in Nebraska (McClure 1949) and 95% ($n = 56$) of lesser prairie-chickens (*T. pallidicinctus*) in Kansas (Robel et al. 2003). Our findings would be complemented by examining bobwhites from Nebraska and Kansas where *O. petrowi* infections have been recorded at high prevalence in pheasants and prairie-chickens. This is the first study to report *O. petrowi* from bobwhites or any Galliformes in Alabama or Virginia.

It is important to distinguish what characteristics of parasitic infections cause disease. As such, we accept Wobeser's (1981) definition that a disease is "any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate, infectious agents, inherent or congenital effects; or a combination of these factors." The effect of eyeworms on bobwhite physiology, behavior, and demographics (e.g., survival, reproduction) is currently being studied, but existing evidence for potential impacts on survival of bobwhites is speculative. Given that no data exist that document whether eyeworm infections alter behavior, we feel hunter-donated birds provide a random and unbiased sample. In other words, there is no reason to believe infected and noninfected individuals are harvested at different rates.

We agree with Olsen et al. (2016) in that surveys, as presented here, provide important information, but experimental approaches will ultimately describe the impacts of helminth infections on populations. For example, Robel et al. (2003) examined relationships of helminth burdens, including *O. petrowi,* on demographics of lesser prairie-chickens in Kansas. Prevalence of *O. petrowi* in lesser prairie-chickens was 95% with a mean of 14 and intensities ranging from 1 to 81. Using telemetry data, they compared clutch size, nest success, movement, home range, and April–November survival between parasitized and nonparasitized birds. There were no significant differences among these demographic parameters. Surprisingly, nest success and April–November survival tended to be greater for parasitized than nonparasitized birds, but the differences were not significant. Thus, albeit there is relatively high prevalence and intensity of eyeworms in the Rolling Plains of Texas, we encourage researchers to take more experimental approaches to determine population-level effects.

We conclude that there is an overall absence of eyeworms in bobwhites across most of the bobwhite's range in the United States. However, it is unknown why some areas support high prevalence and intensities of *O. petrowi* in bobwhites. Thus, reintroduction and translocation programs that seek to introduce individuals to naïve areas of low prevalence should consider infection status of individuals as a precaution to avoid unknown impacts. On the other hand, it may also be noted that migratory birds are also known to be infected by *O. petrowi* (Dunham and Kendall 2014). It is likely that environmental conditions that regulate intermediate host occurrence determine geographic prevalence of *O. petrowi* in bobwhites. We contend that the effects of eyeworm infections are a presently unknown, but intriguing, management concern for bobwhite populations in areas of high prevalence and intensities of *O. petrowi*.

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