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EYEWORMS (*OXYSPIRURA PETROWI*) IN NORTHERN BOBWHITES (*COLINUS VIRGINIANUS*) FROM THE ROLLING PLAINS ECOREGION OF TEXAS AND OKLAHOMA, 2011–13

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ABSTRACT: The Northern Bobwhite (*Colinus virginianus*) has been steadily declining throughout much of its historic range for decades. The Rolling Plains ecoregion of Texas and western Oklahoma, historically rich with wild Northern Bobwhites and one of the last remaining quail strongholds, also has a declining population. During August and October in 2011–13, 348 Northern Bobwhites from the Rolling Plains were examined for eyeworms (*Oxyspirura petrowi*). Of these 348 Northern Bobwhites, 144 (41.4%) were infected with 1,018 total eyeworms. Eyeworm abundance (mean±SE) was 2.9±0.4 (range 0–64), with an intensity (mean±SE) of 7.1±0.6. Eyeworm prevalence was significantly higher in adult Northern Bobwhites (58.7%) than in juveniles (35.4%). Recent research suggests that eyeworms have the potential to cause cellular tissue damage to the eye, but it is unknown how these worms affect host survivability. This study further expands the regional distribution of *O. petrowi* in Northern Bobwhites in the Rolling Plains ecoregion and assesses the prevalence and abundance of infection across host age, host sex, and year. Further research is warranted on the life history of *O. petrowi* and assessing the impacts of eyeworms on their definitive host at individual and population levels.

Key words: *Colinus virginianus*, eyeworm, Northern Bobwhite, Oklahoma, *Oxyspirura petrowi*, quail, Rolling Plains ecoregion, Texas.

INTRODUCTION

The Northern Bobwhite (*Colinus virginianus*) has been declining throughout its native range for decades (Brennan 1991). This decline is especially apparent when it comes to the Rolling Plains ecoregion of Texas and western Oklahoma, which was once regarded for its productive bobwhite habitat. Over the past few decades, much of the focus has been on the effects of habitat loss or degradation due to changes in agriculture practices or variable weather patterns on Northern Bobwhite populations; however, Northern Bobwhite populations continue to decline (Brennan 1991; Bridges et al. 2001). Quail in semiarid environments tend to follow a "boom or bust" population cycle because they fluctuate drastically (Hernández et al. 2007). During summer 2010, a boom year was expected due to above-average precipitation in 2010 (National Oceanic and Atmospheric Administration 2015) and a link between precipitation and Northern Bobwhite population cycle expectations (Bridges et al. 2001). However, the expected Northern Bobwhite population surge of 2010 never came. The missed boom year sparked a research initiative throughout the Rolling Plains of Texas and Oklahoma that investigated disease, contaminants, viruses, and parasites in quail from 2011 to 2013. Early in the study, eyeworms, (*Oxyspirura petrowi*) became the focus because of their spatial and temporal occurrence throughout the Rolling Plains ecoregion.

Oxyspirura petrowi is a heteroxenous parasitic nematode that can be found in the orbital cavity (Addison and Anderson 1969), Harderian and lacrimal glands (Bruno et al. 2015), the nasal sinuses (Dunham et al. 2014),

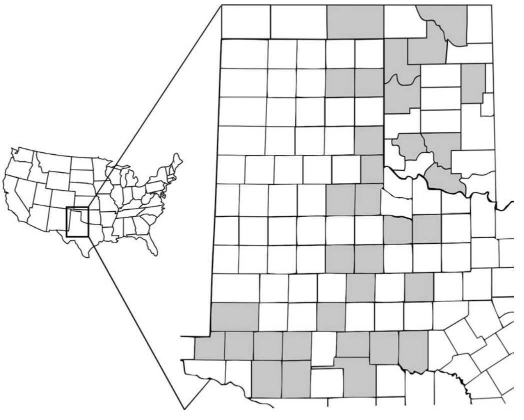

FIGURE 1. Counties in the Rolling Plains ecoregion of Texas and western Oklahoma, USA, sampled for Northern Bobwhites (*Colinus virginianus*), 2011–13.

underneath the eyelids and nictitating membrane (Saunders 1935; Jackson 1969), and inside the lacrimal duct and gland (Robel et al. 2003). Although the life cycle is not completely known, *O. petrowi* has an indirect life cycle and requires an arthropod intermediate host for development. *Oxyspirura petrowi* has been found in many wild avian species (Pence 1972), including the Scaled Quail (*Callipepla squamata*; Landgrebe et al. 2007), Ring-necked Pheasant (*Phasianus colchicus*; McClure 1949), Lesser Prairie-chicken (*Tympanuchus pallidicinctus*; Robel et al. 2003), and Ruffed Grouse (*Bonasa umbellus*; Erickson et al. 1949).

The genus *Oxyspirura* was first documented from 11 counties in the Rolling Plains of Texas from 1961 to 1964 (Jackson and Green 1965). During that study, *Oxyspirura sygmoides* (=*O. petrowi*) was found in 44.1% of 605 Northern Bobwhites and prevalence of infection in adults ranged from 61% to 79% (Jackson and Green 1965). Jackson (1969) speculated on the potentially negative impact of eyeworms on Northern Bobwhite vision. Villarreal et al. (2012) reported similar eyeworm occurrence (57%) over a 3-yr period in Fisher County (Rolling Plains). *Oxyspirura petrowi* was also found in Mitchell County (Rolling Plains) in both the Northern Mockingbird (*Mimus polyglottos*) and Curve-billed Thrasher (*Toxostoma curvirostre*) and at "elevated" levels in Northern Bobwhites and Scaled Quail (Dunham and Kendall 2014; Dunham et al. 2014).

Considering the importance of Northern Bobwhites to the Rolling Plains ecoregion, we provide recent data on the prevalence, abundance, and intensity of *O. petrowi* in Northern Bobwhites; assess prevalence, and abundance across host age, host sex, and year of study; and further evaluate the regional distribution of *O. petrowi* in Northern Bobwhites.

MATERIALS AND METHODS

Study area

In August and October 2011, 2012, and 2013, Northern Bobwhites were trapped in 29 counties throughout the Rolling Plains of Texas and Oklahoma (Fig. 1). Each of the designated trapping teams spent 2.5 d on each of the study ranches or wildlife management areas trapping quail. The Rolling Plains ecoregion is dominated with mesquite (*Prosopis glandulosa*) savannas, junipers (*Juniperus pinchotii*), prickly pear (*Opuntia* spp.), woody species such as lotebrush (*Ziziphus obtusifolia*) and sand shinery oak (*Quercus havardii*), and the grassland species silver bluestem (*Bothriochloa saccharoides*) and buffalo grass (*Buchloe dactyloides*; Rollins 2007). Rangelands comprise about 65% of the Rolling Plains, with croplands making up an additional 30% (Rollins 2007). This ecoregion averages 55.8–76.2 cm of rain annually, and the average temperature is 15–18 C (Texas Parks and Wildlife 2013).

Trapping

Northern Bobwhites were trapped under Texas Parks and Wildlife Scientific Research permits SRP 1098-984 and SRP-0690-152. Northern Bobwhites were handled consistent with Texas A&M University Acceptable Use Policy (2011-193), Texas Tech University Animal Care and Use Committee (11049-07), Texas A&M University-Kingsville (TAMUK) Institutional Animal Care and Use Committee (2009-09-21A), and TAMUK Institutional Biosafety Committee (IBC-ID 009-2011). Twenty galvanized, welded-wire walk-in funnel traps (25.4×91.4×61 cm) were placed near minimally travelled ranch roads on respective trap sites. All traps were covered with vegetation or burlap cloth to provide cover, and each trap was baited using milo (*Sorghum bicolor*) for 2 wk before trapping. Traps were monitored daily at 2 h after sunrise and 1 h before or at sunset. Northern Bobwhites were euthanized in the field by cervical dislocation and placed in a cooler with dry ice and transported to either The Institute of Environmental and Human Health (TIEHH) central receiving laboratory or Buddy Temple Wildlife Pathology and Diagnostic Laboratory at TAMUK.

Eyeworm examination

The eyelids, nictitating membrane, and associated ocular tissue of Northern Bobwhites were thoroughly examined for eyeworms under 1–40× magnification. After the surface of the eyelids and nictitating membrane were examined, the eyelids were removed and the eyeball and its associated ducts and tissues were detached from the orbital cavity and placed into a Petri dish. The Harderian gland, lacrimal gland, and lacrimal duct were removed from the eyeball and teased apart. In addition, 188 heads were individually floated in a jar of 10% physiological saline solution (at room temperature), thereby causing any eyeworms that were still attached to the eye socket area to release and fall to the bottom of the jar. Eyeworms recovered from Northern Bobwhites necropsied at TIEHH (n=188) were placed in saline holding media within a 32 C isotemp CO_2 incubator (model 3550, Fisher Scientific, Pittsburgh, Pennsylvania, USA) and used for additional studies. Eyeworms recovered from Northern Bobwhites necropsied at TAMUK (n=161) were fixed in glacial acetic acid and preserved in 70% ethanol and 8% glycerol. Voucher specimens of *O. petrowi* (107283) from TIEHH were deposited in the US National Parasite Collection, Beltsville, Maryland. Voucher specimens from TAMUK were deposited in the Sam Houston State University Parasite Museum (SHSUP), Sam Houston State University, Huntsville, Texas (SHSUP 000,366–000,381; 131299, 130404, 130644, 130356, 131983, 131479, 131382, 130380, 131461, and 131431).

Data analysis and terminology

Prevalence, mean abundance, mean intensity, and the range of eyeworms within all quail sampled were calculated. Prevalence refers to the number of Northern Bobwhites infected with *O. petrowi* in the sample divided by total quail examined in the sample and mean abundance is the number of *O. petrowi* found in the total sample divided by the total number of quail examined (Bush et al. 1997). Mean intensity is defined as the average number of eyeworms in infected Northern Bobwhites sampled.

Chi-square analysis was conducted to compare prevalence of eyeworms between host sex, host age class, and year sampled (R Development Core Team 2015). *Oxyspirura petrowi* infection data were aggregated toward 0, with a few birds containing most of the parasites, indicating nonnormality. To account for nonnormality, data were fitted with a negative binomial distribution and analyzed using a generalized linear mixed model procedure (PROC GLIMMIX) in SAS 9.3 software (SAS Institute Inc., Cary, North Carolina, USA). Models were created to explain variation in abundance of *O. petrowi* by using independent variables of host age, host sex, and year and their two-way interactions (age×sex, age×year, sex×year). We used backward selection based on type III effects F tests to eliminate terms that did not describe an adequate amount of variation in the response (i.e., parameters that where $P>0.05$). Type III F tests calculate the significance of each parameter after variation attributable to all other parameters in the model has been taken into account. Least-squares mean separation was generated to compare significant effects among the levels of categorical variables (i.e., host age, host sex, and year) and considered means statistically different if $P\leq0.05$. Due to the skewed distribution of the data, our analyses were based on a log (count+1) analysis of variance of a general linear model; back-transformed means and asymmetric SE are presented and referred to as the estimated mean±SE (Sokal and Rohlf 2011). Significance was determined at $P\leq0.05$, and all means are reported as mean±SE.

RESULTS

The eyes, nictitating membrane, and all associated ocular tissue of 348 Northern Bobwhites (97 adults, 251 juveniles; 183 males, 145 females, 20 unknown) were examined for eyeworms. One hundred forty-four (41.4%) were collectively infected with 1,018 eyeworms. Prevalence was similar among male and female Northern Bobwhites (χ^2_1=0.12, P=0.73; Table 1); however, prevalence was higher in adults than juveniles (χ^2_2=15.74, $P<0.0001$). Prevalence also was significantly different (χ^2_2=11.6, P=0.002) among years (Table 1).

Host age, year, and a host age×year interaction best explained *O. petrowi* abundance in Northern Bobwhites. The effect of year was not significant on its own (P=0.640), but it was included in the model because of the host age×year interaction. The effect of host age on abundance of *O. petrowi* depend-

Table 1. Prevalence, abundance (mean±SE), intensity (mean±SE), and range of *Oxyspirura petrowi* in Northern Bobwhites (*Colinus virginianus*) in the Rolling Plains ecoregion of Texas and western Oklahoma, USA, 2011–13.

	Overall	Adult	Juvenile	Male	Female	Unknown	2011	2012	2013
Sample size	348	97	251	183	145	20	41	56	251
Prevalence (%)	41.4	58.7	35.4	40.4	42.1	45	51	21.4	44.2
Abundance	2.9±0.4	5.2±1.1	2.0±0.4	3.1±0.6	2.5±0.6	4.4±11.9	4.6±1.6	2.6±1.2	2.7±0.5
Intensity	7.1±0.6	8.8±1.3	5.8±0.7	7.8±0.9	5.8±0.9	9.6±3.6	9.0±2.0	12.0±2.3	6.2±0.7
Range	0–64	0–61	0–64	0–61	0–64	0–54	0–46	0–61	0–64

ed on year (Fig. 2). In 2011 and 2012, the estimated mean *O. petrowi* was significantly (P=0.012 and P<0.0001, respectively) higher in adults (n=29; 6.2±2.4 and n=10; 13.1±8.8, respectively) compared to juveniles (n=12; 0.8±0.6 and n = 46; 0.3±0.1, respectively) (Fig. 2). However, the estimated mean abundance was similar (P=0.275) in 2013 between adults (n=58; 3.3±1.0) and juveniles (n=191; 2.3±0.4; Fig. 2).

Within adults, there was no difference in estimated mean abundance *O. petrowi* between 2011 and 2012 (P=0.341), 2011 and 2013 (P=0.214), and 2012 and 2013 (P=0.064; Fig. 2). The estimated mean of adults in 2012 (13.1±8.8) and 2013 (3.3±1.0) seemed significantly different; however, when tested in SAS (ESTIMATE) the estimated difference on the back-transformed scale was 3.9±2.8 (95% confidence interval –3.2 to 10). Since the 95% confidence interval includes 0, we accept the null hypothesis of no difference. Within juveniles, there was no difference in the estimated mean between 2011 and 2012 (P=0.180) and between 2011 and 2013 (P=0.146); however, the estimated mean was significantly (P<0.0001) higher in 2013 compared to 2012 (Fig. 2).

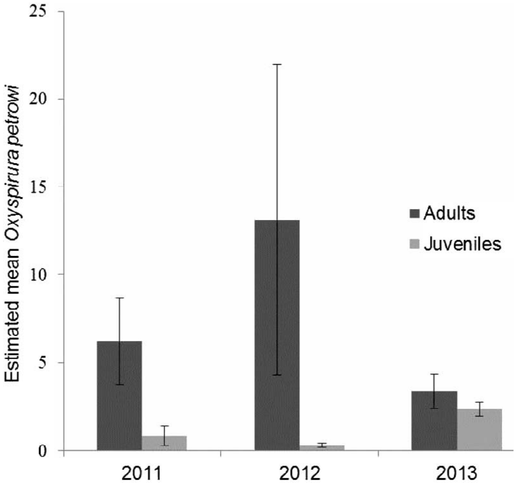

FIGURE 2. Estimated abundance (mean±SE) of *Oxyspirura petrowi* by host age×year predicted by a negative binomial model from 348 Northern Bobwhites (*Colinus virginianus*) collected during August and October 2011–13 within the Rolling Plains ecoregion of Texas and western Oklahoma, USA.

DISCUSSION

Eyeworms were previously documented in 11 counties throughout the Rolling Plains of Texas (Jackson and Green 1965), and the current study expands the survey region to 29 counties throughout the Rolling Plains ecoregion of Texas and Oklahoma. Given the eyeworm occurrence throughout this ecoregion in the early 1960s and with similar distribution in the current study, our study confirms that *O. petrowi* has been persistently enzootic in Northern Bobwhite throughout the region for decades. Baseline studies are needed to determine its presence in other regions of North America, coupled with monitoring to determine whether this nematode spreads to regions where it is absent.

The distribution of eyeworms, although being broad spatially, is disproportionately distributed within age classes of Northern Bobwhites. Two recent studies, both limited to a single county within the Rolling Plains ecoregion, also support our findings that adult

Northern Bobwhites have a higher prevalence and abundance of *O. petrowi* than juveniles (Villarreal et al. 2012; Dunham et al. 2014). In addition, Jackson and Green (1965) found substantially higher prevalence (61–79%) in adult Northern Bobwhites compared to juveniles (16–33%). Prevalence and abundance of parasites can be higher in adults than juveniles due to longer exposure times to infected intermediate hosts (Davidson et al. 1980). In two of 3 yr of our study, we also found differences in abundance due to host age. It is possible that no differences by host age in 2013 may be related to increased rainfall during summer 2013. Peak transmission of many heteroxenous life cycle nematodes typically coincides with the wet season when intermediate hosts are most plentiful (Davidson et al. 1980). We speculate that increased precipitation from 2012 to 2013 triggered an increase in vegetation, facilitating intermediate host survival and increasing transmission potential to the definitive host. Within age classes, we saw little difference in infection across years, indicating that prevalence of infection among adults remains consistent temporally. Drought conditions persisting in 2012 may have affected host and parasite recruitment and survival during breeding season, resulting in lower eyeworm prevalence in juveniles in 2012.

Due to the microhabitats where adult *O. petrowi* occurs, we speculate that infections have the potential to impair respiratory function, cause visual obstruction, increase energy expenditure, and reduce flight and forage ability. Additional research is needed to understand the impact, if any, *O. petrowi* has on the survivability of Northern Bobwhites at the population level before drawing conclusions on the status of this nematode as a potential factor in quail decline. The results of this study provide recent information on the spatial, temporal, and demographic distribution of *O. petrowi* in Northern Bobwhites from the Rolling Plains ecoregion. In addition, this research identifies *O. petrowi* as a common and frequently occurring helminth of Northern Bobwhites within the study area.

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