Plains lubber grasshopper (*Brachystola magna*) as a potential intermediate host for *Oxyspirura petrowi* in northern bobwhites (*Colinus virginianus*)

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SUMMARY

Oxyspirura petrowi is a heteroxenous parasitic nematode that has been reported in high prevalences from birds in the Order Galliformes experiencing population declines in the USA. There is a paucity of information regarding the natural history *O. petrowi*, including the life cycle and effects of infection on wild bird populations. In order to study the life cycle of this parasite, we collected plains lubber grasshoppers (*Brachystola magna*) from a field location in Mitchell County, Texas. We found third-stage larvae (L3) in 37.9% (66/174) *B. magna*. We determined that they were *O. petrowi* through morphological comparison of L3 from experimentally infected *Acheta domesticus* and by sequence analysis. Then, we showed that *B. magna* are a potential intermediate hosts for *O. petrowi* infections in northern bobwhites (*Colinus virginianus*) in a laboratory setting by experimental infection. We first detected shedding of eggs in feces using a fecal float technique 52 days post infection. In addition, we recovered 87 *O. petrowi* from experimentally infected northern bobwhites. Although we detected shedding in feces, recovery of eggs was low (>5 eggs/g). Future work is needed to understand shedding routes and shedding patterns of northern bobwhites infected with *O. petrowi*.

Key words: birds, Galliformes, grasshopper, intermediate host, life cycle, northern bobwhite, Orthoptera, *Oxyspirura petrowi*.

INTRODUCTION

Parasites of the genus *Oxyspirura* (Spirurida: Thelaziidae) are heteroxenous nematodes that primarily use avian species as definitive hosts (Addison and Anderson, 1969). *Oxyspirura* spp. have been reported from more than 80 avian species worldwide (Addison and Anderson, 1969). Despite many published records of *Oxyspirura* spp. in bird species across the globe, very little is known about their life cycle. Of the approximately 72 species of *Oxyspirura* reported, the life cycle is only known in one species that infects birds, *Oxyspirura mansoni*.

The life cycle of *O. mansoni* was described by three separate studies in the 1920s (Fielding, 1927; Kobayashi, 1927; Sanders, 1928). The definitive host was determined to be the chicken (*Gallus gallus*) and the intermediate host is the Surinam cockroach (*Pycnoscalus surinamensis*; Dictyoptera: Blattodea). Sexually mature *O. mansoni* are found under the nictitating membranes, in conjunctival sacs, and nasal-lacrimal ducts of chickens (Schwabe, 1951). Eggs are

passed through the nasal-lacrimal ducts into the mouth and then swallowed before being passed in the feces (Fielding, 1927). Cockroaches are infected from ingesting eggs or recently hatched first-stage larvae (L1) and *O. mansoni* develop into infective third-stage larvae (L3) in approximately 50 days (Fielding, 1927; Schwabe, 1951). A similar life cycle can be inferred with *O. conjunctivalis* using the speckled cockroach (*Nauphoeta cinerea*; Dictyoptera: Blattodea) as an intermediate host and primates as definitive hosts (Ivanova *et al.* 2007); however, complete time for development was not analysed.

Recently *Oxyspirura petrowi* has received attention due to the high prevalences detected in native Galliformes species with declining populations in the USA (Robel *et al.* 2003; Dunham *et al.* 2014). These population declines are multi-factorial and include habitat loss and fragmentation, climatic variables and other factors (Johnson *et al.* 2004; Parent *et al.* 2016; Rho *et al.* 2015). The direct impacts of *O. petrowi* on wild populations are currently unknown; however, a histopathology study has described lesions present on the cornea, lacrimal gland and Harderian gland in infected birds (Bruno *et al.* 2015). These lesions indicate that there is the potential for *O. petrowi* to affect the eyesight of infected birds, which could affect their

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ability to forage or escape predators. Although *O. petrowi* has been detected in several species of birds, no studies have looked for intermediate hosts (Pence, 1972, 1975; Dancak *et al*. 1982; Robel *et al*. 2003, 2005; Calegaro-Marques and Amato, 2013). The objectives of this study were to: (1) identify a potential intermediate hosts for *O. petrowi* infection in northern bobwhites (*Colinus virginianus*) in Western Texas; (2) establish experimental infection methods for *O. petrowi* in northern bobwhites; and (3) determine time for development of *O. petrowi* L3 to adults in experimentally infected northern bobwhites.

MATERIALS AND METHODS

Field sampling

We focused our sampling on plains lubber grasshoppers (*Brachystola magna*) based on a pilot study indicating they were naturally infected with *O. petrowi*. We identified *B. magna* using an online key (Brust *et al*. 2014). We collected *B. magna* using sweep nets and by hand from a field location in Mitchell County, Texas from 12 May 2015 to 28 August 2015. This location was chosen because of previous and ongoing research showing current *O. petrowi* infection in northern bobwhites (Dunham *et al*. 2014). After collection, grasshoppers were stored in aerated containers and transported to the laboratory for dissection.

Larvae collection

We euthanized grasshoppers by placing them at 4 °C then decapitating them with a pair of scissors. We then dissected the grasshoppers, pinned the grasshoppers open, and examined the grasshoppers under a dissecting microscope for free moving larvae and larvae still in capsules, as previously reported for the development of *O. mansoni* and *O. conjuctivalis* in intermediate hosts (Sanders, 1928; Ivanova *et al*. 2007). After we examined the grasshoppers for larvae, we then placed the grasshoppers into a Petri dish containing 1/4 Ringer's solution (Sigma-Aldrich LLC) and incubated for approximately 30 min to 1 h at 37 °C (Fielding, 1927). We then examined the contents of the Petri dish under a dissecting microscope for free moving larvae. We collected larvae using a pipette for use in morphological and molecular analysis. Briefly, all L3 were examined under a dissecting microscope for to ensure collected L3 were morphological similar to each other. Then we collected two L3 from each infected grasshopper for molecular identification. We used the remaining L3 for morphological identification and experimental infections. In addition, we collected the intestines and reproductive tract from each grasshopper after incubation and placed them into a 2 mL microcentrifuge tube for DNA extraction and subsequent molecular analysis.

Experimental infection of Acheta domesticus

To be able to compare the morphology of L3 from grasshoppers caught in the field to *O. petrowi* L3, we experimentally infected 30 *A. domesticus* (house crickets; PetsMart Inc., Phoenix, AZ, USA) with gravid female *O. petrowi* containing eggs with visible L1. Briefly, *A. domestics* were stored individually in $152.4 \times 76.2 \times 76.2$ mm^3 containers with a mesh cover and food deprived for 12 h. Then we placed a depressed glass slide containing one gravid female *O. petrowi* in water in each container until the cricket fed on the nematode. We assumed the nematode was ingested by the cricket if no evidence of the nematode was found on the slide or in the container with the cricket. After feeding on *O. petrowi,* we placed the cricket on Fluker's Farms Orange Cube diet (Fluker's Farms, Port Allen, LA, USA) until they were dissected or died. We dissected *A. domesticus* and collected L3, intestines, and the reproductive tracts as described above for *B. magna*. It is important to note that we could not verify *A. domesticus* were not infected prior to our infection; however, we feel this case is highly unlikely. *Acheta domesticus* used in this study were raised in captivity and likely had little or no contact with bird feces contaminated with *O. petrowi* eggs.

Identification of larvae and molecular testing of Brachystola magna

We compared the morphology of L3 collected from both *B. magna* and *A. domesticus* by clearing the larvae in 5% glycerin and 95% ethanol solution and observing under 100× using EVOS XL microscope (Thermo Fisher Scientific Inc.). We took measurements of larvae from photographs using Gimp v2.8 (Gimp developing team). In addition to using morphology for identification of L3 from *B. magna*, we used PCR and sequencing to aid in our identification.

We extracted DNA from L3, *B. magna* and *A. domesticus* using Qiagen DNeasy blood and tissue kits (Qiagen, Germantown, MD, USA). We included a negative extraction sample of PBS every 12th sample. The PCR reaction was done at 25 μL volumes with the primers OXY_ITS2F (5′-CTTAGCGGTGGATCACTTGG-3′) and the previously published primer QEW_2578R (Xiang *et al*. 2013). These primers were designed to amplify a 244 base pair region of the ITS2 locus. Each reaction included 5 μL of DNA in 20 μL of master mix containing 11 μL of H$_2$O, 1.5 mM MgCl$_2$, 0.4 mM each dNTP (Promega, Madison, WI, USA), 0.5 μM each primer, and 1.25 U of GoTaq® DNA Polymerase (Promega). Cycling

parameters were 95 °C for 3 min followed by 20 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 45 s and a final extension of 72 °C for 10 min. We then analysed PCR products on a 1.75% agarose gel and we sent 29 PCR products (one L3 from *A. domesticus* and 28 L3 from different *B. magna*) to Molecular Cloning Laboratories (MCLAB LLC, South San Francisco, CA, USA) for bidirectional sequencing. Chromatogram data were analysed using Sequencher v 5.1 (Gene Codes, Ann Arbor, MI, USA). We compared sequences from this study to previously published sequences using the blast function available through the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA). In instances where only one L3 was recovered, we used it for molecular testing.

Experimental infections of northern bobwhites

To determine whether *B. magna* were suitable intermediate hosts for *O. petrowi* in northern bobwhites, we conducted two experimental infection trials. In the first trial, we fed four, previously uninfected, pen-raised northern bobwhites *B. magna* that contained free moving L3. Prior to feeding, we determined *B. magna* were infected by partial dissection. Each bird was fed one grasshopper (without head and legs); however, we were unable to accurately quantify the larvae in each grasshopper. We euthanized grasshopper infected birds; one bird at 30 days post infection (dpi); one bird at 44 dpi; one bird at 45 dpi and one bird at 70 dpi. After euthanasia, we dissected the birds and collected nematodes from the orbital cavity as previously described (Dunham *et al*. 2014). We then morphologically identified nematodes found in the orbital cavity of the infected northern bobwhites as *O. petrowi* based on: undivided buccal capsule; oesophagus not clearly divided into muscular and glandular portions; unequal spicules; and absence of gubernaculum (Addison and Anderson, 1969; Pence, 1972). We also amplified a partial 244 base pair region of the ITS2 locus using the same PCR described above.

In order to control the dose of infection, we collected L3 from *B. magna* to examine another infection method. Briefly, L3 were collected by pipette from Petri dishes after incubation and placed into a 2 mL microcentrifuge tube. We then infected five northern bobwhites: one with ten larvae; two with 20 larvae; and two with 40 larvae in 400 μL of Ringer's solution by oral gavage. All experimental infections were done the same day L3 were collected. We inoculated two additional birds with 400 μL Ringer's solution to act as uninfected negative controls. We selected these infection doses because they are representative of worm burdens observed in wild caught northern bobwhites (Dunham *et al*. 2014). We did not euthanize any of the gavage infected birds because they are being used in a larger ongoing study. This was done to reduce the overall number of birds used in experimental trials. We monitored birds for 90 min post-infection to ensure they did not regurgitate the grasshoppers or the gavage and monitored birds daily throughout the study for any clinical signs of disease (ruffled feathers, lethargy, ataxia, diarrhoea, etc.). We collected fecal samples from each bird daily to detect egg shedding using a fecal float procedure using the Cornell–Wisconsin egg-counting double centrifugation procedure (Zajac *et al*. 2012) with a magnesium sulphate (MgSO$_4$; specific gravity = 1.31) fecal float solution. Briefly, we mixed the fecal sample with deionized water, poured through a 200 μm sieve into a 15 mL conical tube, and centrifuged for 5 min at 500 g. We then poured off the supernatant and resuspended the pellet in MgSO$_4$ and vortexed. Next we centrifuged the sample for 5 min at 500 g with the centrifuge brake turned off. After letting the coverslip remain on the tube for 5 min, we carefully examined each field of the slide under 100×. We included fecal samples through 70 dpi.

Statistical analysis

We analysed the prevalence of PCR positives by month with logistic regression using robust standard errors and we analysed L3 burden in grasshoppers by negative binomial regression using robust standard errors. We considered a sample PCR positive if either the grasshopper was PCR positive or if a collected L3 from the grasshopper was PCR positive. We conducted analyses using Rv3.2.4 (www.r-project.org) using the glm() and glm.nb() functions and the package sandwich v2.3 for implementing robust standard error estimates.

RESULTS

Brachystola magna natural infections

We detected L3 in 37.9% (64/174) of *B. magna* by dissection and incubation. The first L3 we detected in naturally infected *B. magna* was on the 17th of June. Based on PCR, we detected an additional seven *B. magna* infected with *O. petrowi*, with the earliest being detected on the 13th of May. We did not detect any L3 in these seven *B. magna* based on dissection or incubation. The highest PCR prevalence and L3 burden of *O. petrowi* infections in *B. magna* were in August and lowest PCR prevalence and L3 burdens were in May (Table 1). During dissection of *B. magna*, we detected L3 in capsules along the body wall (Fig. 1) and moving freely throughout the body cavity. Based on dissection alone, we detected L3 in 17.8% (31/174) of *B. magna*; however after incubation, we detected L3 from an additional 33 *B. magna*. The average L3 recovered from infected grasshoppers was 9.8 L3/

Table 1. Results of logistic regression of PCR prevalence by month and negative binomial regression of third-stage larvae burden by month.

Month	Grasshoppers infected with third-stage larvae/ grasshoppers sampled (%)[a]	Coefficients ± standard error Odds ratios (95% confidence intervals)	Third-stage larvae/ infected grasshopper Mean (range)	Coefficients ± standard error Incidence rate ratios (95% confidence intervals)
May	1/23 (4.3)	−2.14 ± 0.6 0.1 (0.0–1.3)	0/0 0	−19.8 ± 0.6*** 0
June	5/18 (27.7)	Referent[b]	26/4 6.5 (3–13)	Referent
July	23/70 (32.9)	0.2 ± 0.7 1.2 (0.3–4.8)	94/18 5.2 (1–21)	−0.2 ± 0.7 0.8 (0.2–3.3)
August	44/63 (69.8)	2.1 ± 0.7** 8.2 (2.1–32.2)	532/44 12.1 (1–90)	1.6 ± 0.6** 5.2 (1.6–16.9)

***$P = 0$.
**$P < 0.001$.
[a] Includes seven PCR positive samples one in May, one in June, and five in July. We did not recover any third-stage larvae from these grasshoppers.
[b] Comparison group.

grasshopper (9.0–10.6 95% CI; range 1–90). We did observe 'sausage-like' larvae similar to the description of second stage *Thelazia callipaeda* described by Otranto *et al.* (2005); however, we were not able to amplify *O. petrowi* DNA from those samples and therefore did not include them in our analyses.

Acheta domesticus experimental infections

We did not find evidence of the gravid *O. petrowi* in all 30 cages and therefore assumed all *A. domesticus* fed on the nematode. Seven *A. domesticus* died within 7 dpi. We subsequently dissected and ran PCR on these *A. domesticus*, but did not detect

Fig. 1. Encapsulated third-stage larvae in *Brachystola magna*. Capsules ($n = 3$) are marked with arrows. Scale bar 20 mm.

evidence of *O. petrowi* infection in any of the seven dead *A. domesticus*. We euthanized and dissected two *A. domesticus* each on 10, 12, 14, 17, 30 and 40 dpi. We did not observe any larvae during the dissection, but did get two PCR-positive result from one *A. domesticus* from 12 and 30 dpi. On 45 dpi, we recovered two L3 (based on well-developed intestinal tract and presence of genital stem cells) from one *A. domesticus*. The remaining *A. domesticus* were euthanized and dissected on 47 dpi ($n = 4$) and 48 dpi ($n = 5$). We found an additional three *A. domesticus* infected and recovered a total of eight L3. Including the PCR positive, the infection prevalence was only 16.6% (5/30) from all infected *A. domesticus*. We obtained PCR-positive results from six L3 and sequenced one PCR positive which had a 99% identity to previously published *O. petrowi* ITS2 sequences (GenBank# KF110800, KF110799 and KF306222). We used the remaining four L3 for morphological comparison with L3 from *B. magna*.

Identification of larvae

We did not detect any significant differences in any of the morphological measurements between L3 collected from *A. domesticus* and *B. magna* (Table 2). In addition, we obtained at least one PCR positive from L3 collected from each grasshopper. Furthermore, sequence analysis from 28 L3 samples from *B. magna* (GenBank# KT958840–KT958867) revealed a 99–100% similarity to previously published *O. petrowi* ITS2 sequences (GenBank# KF110800, KF110799 and KF306222).

Northern bobwhite experimental infections

All birds were presumed negative for *O. petrowi* infection prior to feeding based on fecal float and all
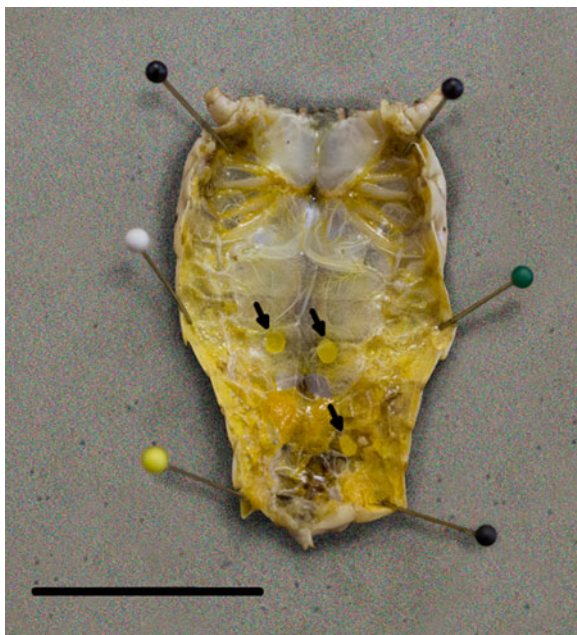
Table 2. Morphological characteristics from L3 *Oxyspirura petrowi* larvae recovered from *Acheta domesticus* (*n* = 4) and *Brachystola magna* (*n* = 24).

Measurement	*Acheta domesticus* (95% confidence interval)	*Brachystola magna* (95% confidence interval)
Length	3.11 (3.03–3.19)	3.14 (2.95–3.34)
Width	0.10 (0.06–0.14)	0.10 (0.09–0.11)
Oesophagus length	0.23 (0.19–0.27)	0.28 (0.26–0.30)
Distance of nerve ring from Anterior end	0.08 (0.07–0.09)	0.09 (0.08–0.10)

All measurements in mm.

birds were infected with an unidentified strongylid from the breeder (Fig. 2F). On 30 dpi, we euthanized one bird and found 23 *O. petrowi* (13 females and 10 males) in the lacrimal duct and under the nictitating membrane. Morphological examination indicated *O. petrowi* were not sexually mature with male's spicule development incomplete and females with no egg development in the reproductive tract (Fig. 2A and C). We collected six females and four males and 22 males and 25 females from the birds we euthanized 44 and 45 dpi, respectively. In these nematodes, we detected morulated eggs in the uteri of the females (Fig. 2D) and spicule development was complete in males (Fig. 2B). We recovered seven adult *O. petrowi* from the bird we euthanized on 70 dpi. We observed that both males (*n* = 5) and females (*n* = 2) were fully developed and eggs containing L1 were visible in the reproductive tract of the female (Fig. 2B and E). We detected one PCR positive from at least one *O. petrowi* recovered from these experimentally infected birds.

We first detected egg shedding at 52 dpi (*n* = 3) and all infected birds, which were not euthanized, shed eggs by 56 dpi (Fig. 2F). The one remaining bird infected by feeding *B. magna* shed at 55 dpi. The average mass of feces used for fecal float analysis was 3.7 g (3.5–3.9 95% CI). The number of eggs detected with fecal floats throughout the study was low 2.2 eggs/g of feces (1.2–3.2 95% CI). In order to determine if the poor shedding detection was a result of eggs not passing entirely through the intestinal tract, we collected the contents of the crop, ventriculus, proventriculus, small intestine and cecum in separate containers from the bird euthanized 70 dpi. We then performed a float using $MgSO_4$, but we were unable to detect any eggs from the intestinal contents. Finally, we did not detect the presence of blood in the oesophagus or intestinal tract of any of the *O. petrowi* collected during this study.

DISCUSSION

Understanding the natural history and life cycle of *O. petrowi* is an important step in understanding how these nematodes affect wild bird populations. This allows for experimental infection methods that more accurately simulate how infection occurs in wild bird populations in a controlled laboratory setting. Experimental infections increase our understanding of the effects of infection on individual birds and aids in the development of diagnostic tests (quantitative PCR) that can be used to determine infection status in wild birds. In this study, we showed *B. magna* populations are naturally infected with *O. petrowi* in a high prevalence (>35%). Furthermore, we demonstrated that *B. magna* are suitable intermediate hosts for *O. petrowi* in northern bobwhites through experimental infection. Although our data suggests *B. magna* are suitable intermediate hosts for *O. petrowi* in a laboratory setting, confirming *B. magna* as the primary intermediate host in a natural setting was outside the scope the current study.

Insects in the order Orthoptera are known to be important components of northern bobwhite diets (Parmalee, 1953; Rollins, 1980); however, to our knowledge, no studies have recorded *B. magna* being used as a food source by northern bobwhites. The majority of studies that have analysed diets in northern bobwhites do not report arthropod genera, but simply report insect food sources as animal matter or only identify them to order (Wood *et al.* 1980; Harveson *et al.* 2004). We believe it is possible that northern bobwhites can utilize *B. magna* as a food source because: unlike other lubber grasshoppers *B. magna* that are non-toxic to vertebrates (Burleson, 1974; Bright *et al.* 1994); other large grasshopper species of similar size (e.g. *Schistocerca americana* and *Melanoplus spretus*) have been reported in diet studies of quail (Judd, 1905); and the range of *B. magna* overlaps with northern bobwhites (Brust *et al.* 2014). These qualities may make them a potential food source for northern bobwhites, particularly when females are nesting and their protein requirements are high, but foraging time is brief (Host and Physics, 2015). The perceived increased L3 burden of infected *B. magna* in August may be related to the increased time for development and development to L3 later in the year, which would make them easier to detect.

Our experimental infection and the resulting development of *O. petrowi* in *A. domesticus* are hard to interpret. On one hand, we were only able to detection *O. petrowi* infection in 5/30 experimentally infected *A. domesticus* and our L3 burden was lower than in naturally infected *B. magna*. However, we do not know if this is a result of *A. domesticus* being poor intermediate hosts or a result of an inadequate infection method. In a natural
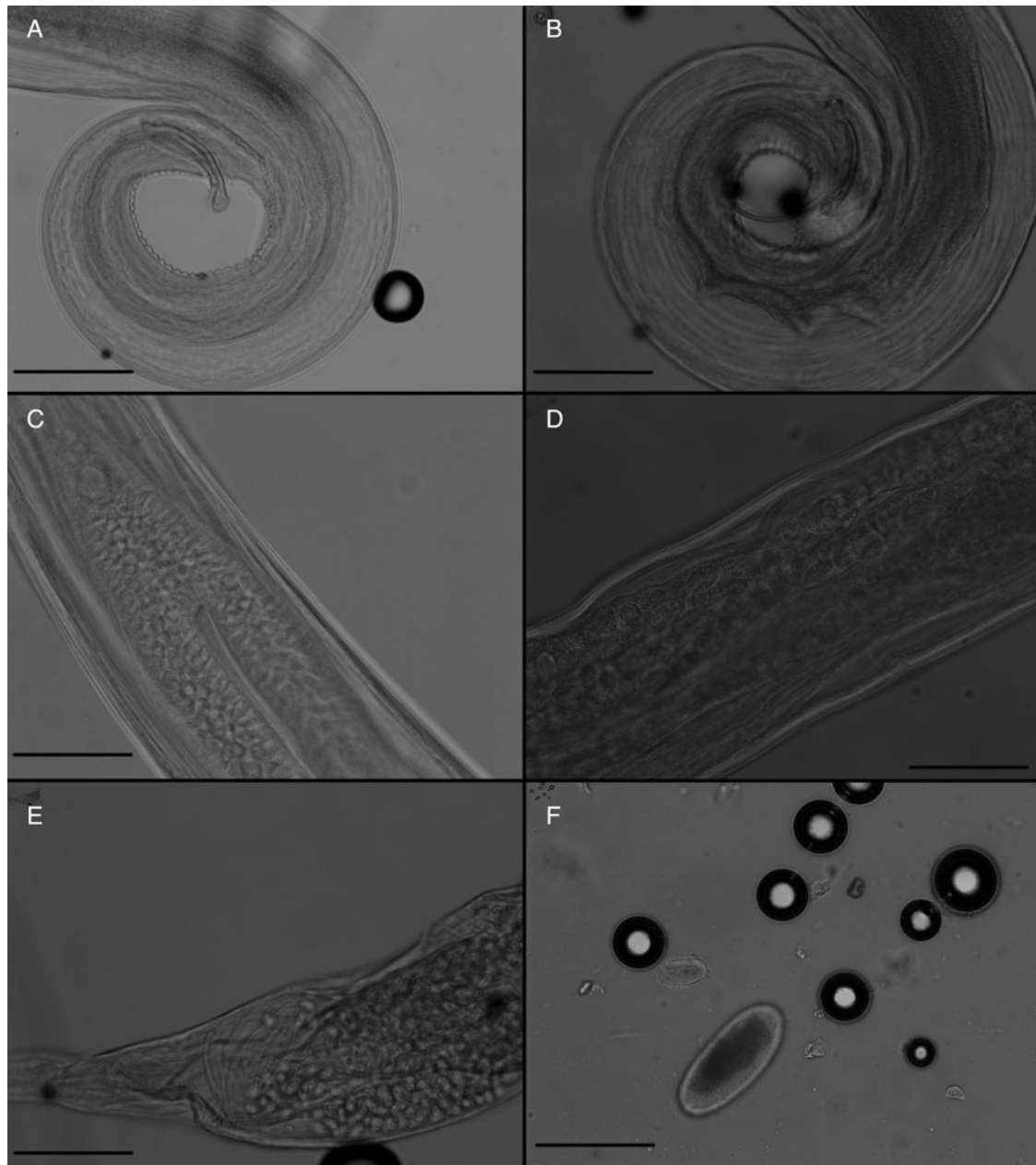
Fig. 2. Comparison of *Oxyspirura petrowi* recovered from experimentally infected northern bobwhite (*Colinus virginianus*): (A) male *O. petrowi* 30 dpi. Short spicule green arrow and long spicule black arrow; (B) male *O. petrowi* 70 dpi. Short spicule green arrow and long spicule black arrow; (C) female *O. petrowi* 30 dpi, note absence of eggs in reproductive tract; (D) female *O. petrowi* 45 dpi, note morulated eggs (arrows) in the reproductive tract; (E) female *O. petrowi* 70 dpi, note first-stage larvae in eggs (arrows) in the reproductive tract; (F) *O. petrowi* egg marked with an arrow next to unidentified Strongylid species egg. Scale bars for A, B, E and F are 100 and 50 μm for C and D.

infection, *O. petrowi* eggs would be shed by and then passed in feces, which would permit longer time for egg development than our infection method. Previous research on *O. mansoni* has shown the average size of embryonated eggs collected from feces are larger than those collected from the vagina and uteri of gravid female worms, which could support the need for further development before they become infectious. However, experimental infection of Surinam cockroaches with eggs collected from gravid *O. mansoni* females resulted

the development of enough larvae to describe the development in intermediate hosts (Schwabe, 1951); although, levels of infection were not reported.

The morphology of L3 *O. petrowi* are similar to the descriptions of other L3 from within Thelaziidae. The best described examples are *T. callipaeda* in flies from the genus *Phortica*, *O. mansoni* from Surinam cockroach and *O. conjunctivalis* from *Nauphoete cinerea* (Schwabe, 1951; Otranto *et al.* 2005; Ivanova *et al.* 2007). Although we did observe 'sausage-like' larvae similar to the description of

second stage *T. callipaeda* (Otranto *et al.* 2005), we were unable to verify any stage larvae were *O. petrowi* other than L3.

Our detection of eggs from feces at 52 dpi suggests development of *O. petrowi* is consistent with *O. mansoni* in chickens, which shedding in feces was detected 60 dpi (Sanders, 1928). The low levels of eggs detected by fecal flotation were an unexpected result. This low rate of shedding may indicate infection in the laboratory is not an accurate representation of a naturally occurring infection or *Oxyspirura* spp. shed low levels of eggs. In supporting the later, several previous experimental and field studies on *O. mansoni* in chickens have reported difficulty in collecting eggs from the feces and throughout the digestive tract (Fielding, 1927; Sanders, 1928; Schwabe, 1951).

There are other potential explanations for our low recovery of eggs from fecal samples. First, our fecal flotation technique may have not been sensitive enough. We do not think this is the case. Although other flotation techniques perform better when there is a high level of eggs (>100 eggs/g), the Cornell–Wisconsin double centrifugation technique has a high reported sensitivity (>90%) for low egg/g (<7 eggs/g) counts (Egwang and Slocombe, 1981, 1982). Furthermore, the Cornell–Wisconsin method is recommended for samples were egg recovery is expected to be low, but the more commonly used McMaster technique and its variants have a higher analytical sensitivity (25 eggs/g; Zajac *et al.* 2012).

Another potential explanation for low egg recovery is the result of the immune response associated with the pen-raised northern bobwhites having an infection with an unidentified stongylid. The effects of multiple parasitic infections on hosts are largely understudied, but in wild animals multiple infections are considered the rule rather than the exception (Bordes and Morand, 2011). Although it is understudied, polyparasitism has been reported from northern bobwhites with no reported detrimental effects (Davidson *et al.* 1980, 1991). Therefore, the dual infection in our study could potentially be a closer representation to naturally infected northern bobwhites; however, effects of polyparasitsm are known to range from antagonistic to synergistic depending on the hosts and parasites involved (Tefler *et al.* 2010) and because all of our experimental infected birds had dual infections we were unable to evaluate if there was an effect on *O. petrowi* egg shedding.

Two previous studies on *O. petrowi* reported that blood was readily observed within the body of *O. petrowi* removed from wild quail species (Dunham *et al.* 2014, 2015). In our present study, we did not detect similar findings from any of the nematodes collected from experimentally infected birds. This may be related to *O. petrowi* only feeding on blood during certain times of its life cycle; however, we collected *O. petrowi* during different stages of development, including gravid females. Blood feeding at certain times in nematode development has been reported with *Litomosoides sigmodontis* (Attout *et al.* 2005) and we did not collect the samples to rule this possibility out for *O. petrowi*.

The results of this study add a valuable piece of information in our understanding of *O. petrowi* infections in northern bobwhites. These data suggest a potential model intermediate host species, which can be used to monitor how anthelminthic treatment of northern bobwhites affects the availability of *O. petrowi* in intermediate hosts. Furthermore, it provides an experimental infection technique that allows us to analyse the impacts *O. petrowi* infection has on individual birds, as well as, providing a means to perfect diagnostic tools (e.g. quantitative PCR) for identifying infection in wild birds antemortem. Future research is needed to determine the factors which influence shedding of *O. petrowi* eggs in northern bobwhites as well as the best biological samples to collect from birds for diagnostic assays.

CONFLICTS OF INTEREST

None.

ETHICAL STANDARDS

All animal use procedures were approved by the Texas Tech University Institutional Animal Care Use Committee (Protocol no. 13066-08).

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