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Source: Journal of Parasitology, 101(1):98-101.
Published By: American Society of Parasitologists
DOI: http://dx.doi.org/10.1645/13-448.1
URL: http://www.bioone.org/doi/full/10.1645/13-448.1

J. Parasitol., 101(1), 2015, pp. 98–101

Live Eyeworm (*Oxyspirura petrowi*) Extraction, In Vitro Culture, and Transfer for Experimental Studies

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ABSTRACT: Northern bobwhite (*Colinus virginianus*) have experienced a dramatic decline in West Texas over the last 3 yr, and investigations are underway to evaluate the role of parasites in this decline. One of the key parasites being investigated is the eyeworm (*Oxyspirura petrowi*). Live eyeworms were extracted from both live and dead northern bobwhites, and in vitro survival was tested using 10 liquid media. Eyeworms placed in an egg white and physiological saline solution lived for at least 36 days. Live *O. petrowi* placed into the eyes of uninfected pen-raised bobwhites were monitored for 21 days to demonstrate successful transfer. Eyeworm behavior during feeding, mating, and development were monitored. This study is important to research that requires "banking" of live *O. petrowi* from wild-captured definitive hosts for life history studies and assessing the impact of *O. petrowi* on host individuals.

The long-term decline in northern bobwhite (*Colinus virginianus*) populations throughout North America has prompted interest in re-examining population regulating factors that have been previously considered inconsequential. One of the factors that is often overlooked or not thoroughly examined is the role that parasitic helminths may play in negatively impacting host populations. The eyeworm (*Oxyspirura petrowi*) has recently received attention from researchers concerned about the decline of the bobwhite (Villarreal et al., 2012; Xiang et al., 2013). With concerns rising, this study provides important behavioral characteristics and life cycle information of the eyeworm that were previously unknown.

Although there is a substantial volume of literature on the eyeworm *Oxyspirura mansoni* from the early 20th century (Ransom, 1904; Fielding, 1926; Sanders, 1928; Schwabe, 1951), likely due to its negative impact on the poultry industry, little is known about the life history or potential negative impacts of *O. petrowi* in wild birds. Ruff (1984) suggested that the life cycle of *O. petrowi* is likely similar to that of *O. mansoni*. However, it has yet to be described as to which arthropod species serve as intermediate hosts, how infective larvae are maintained in intermediate hosts, how larvae exit the intermediate host when ingested by the definitive host, and where larval and adult stages occur in the definitive host for *O. petrowi*.

Studies based on visual observation have found little evidence of pathological responses caused by *O. petrowi* (McClure, 1949; Pence, 1972), whereas ophthalmia or eye inflammation progressing to destruction of the eyeball has been noted for *O. mansoni* in poultry (Ruff and Norton, 1997). Presently, no in-depth histological examinations have been performed. In addition, there have been no studies examining the potential host behavioral or physiological impacts caused by *O. petrowi* in bobwhites. However, Robel et al. (2003) reported lesser prairie-chickens (*Tympanuchus pallidicinctus*) that had elevated numbers of *O. petrowi* tended to be slightly underweight. Consequently, there is a need to better understand the life history of *O. petrowi* and to determine its potential impact on its definitive host through controlled experimental studies. The objectives of this study were to determine whether live *O. petrowi* can be successfully removed from northern bobwhites (definitive host), maintained in vitro, and transplanted into uninfected bobwhites.

DOI: 10.1645/13-448.1

Northern bobwhites were live-trapped from June 2013 to September 2013 on a 120,000-ha cattle ranch (32°10.28′N, 101°0.91′W) in Mitchell County, Texas, and transported to The Institute of Environmental and Human Health Aviary at Texas Tech University. Each bobwhite was individually housed in 25 × 61-cm quail breeding battery (G.Q.F Manufacturing Co., Savannah, Georgia). Bobwhites were trapped and handled under Texas Parks and Wildlife permit SRP-1098-984, Texas A&M University AUP 2011-193, Texas Tech University ACUC 11049-07, and Texas Tech University ACUC 13027-03. Pen-raised adult northern bobwhites purchased from The Quail Ranch of Oklahoma (Wardville, Oklahoma), a USDA and National Poultry Improvement Plan–approved breeder, were used in the live eyeworm transfer experiment. Thirty days before *O. petrowi* transfers, pen-raised bobwhites were gently restrained manually and medicated with the topical parasiticide VetRx (Goodwinol Products Corp., Pierce, Colorado), using the manufacturer's dosing recommendations, to ensure each bird was infection free. Voucher specimens of *O. petrowi* (107283) were deposited in the U.S. National Parasite Collection, Beltsville, Maryland.

The extraction of live *O. petrowi* from wild-captured bobwhites was conducted by manually restraining and giving a topical anesthetic (0.5% Proparacaine HCl Ophthalmic solution; Akorn Inc., Lake Forest, Illinois) to reduce potential discomfort or irritation. A paintbrush hair was laid over the eye, and reactivity was monitored to ensure that the bobwhite's eyes were fully anesthetized. Once the eye was fully anesthetized, forceps (Sontec 14–4340; Sontec Instruments, Centennial, Colorado) were lightly lubricated with GenTeal gel (clear soothing eye drops; Novartis Ophthalmics, St. Louis, Missouri) to minimize any chance of corneal ulcerations and then used to examine the eyes. Eyelids, upper and lower, were gently lifted to examine for the presence of eyeworms. Next, the nictitating membrane was gently pulled over the eye, and slowly manipulated up and down, to find and/or initiate movement of any eyeworms that were not previously found. Since eyeworms evade forceps, the use of a magnifying ocular headset (DA-5 OptiVisor headband magnifier, ×2.5 magnification, 20-cm focal length; Donegan Optical, Lenexa, Kansas) and a light source helped aid in eyeworm collection. Eyes were flushed with balanced salt solution (Alcon Laboratories, Fort Worth, Texas) using a 22-gauge irrigation cannula if eyeworms could not be extracted using forceps. Eyeworms were placed in physiological saline solution at 37 C (Schwabe, 1951) until they were divided into their respective media. Bobwhite eyes were examined for 3 consecutive days after eyeworm removal by applying 2 drops of fluorescein eye stain (Ful-Glo, NDC-17478-404-01; Akorn Inc.) to each eye to determine whether the procedure produced lacerations or lesions to the eyelid, eye surface, and nictitating membrane. The extracted eyeworms were used for both the transfer of eyeworms to 9 uninfected pen-raised northern bobwhites and to study their survivability to various media.

The transfer of 54 eyeworms to 9 pen-raised northern bobwhites happened within 30 min after removing the eyeworms from wild-captured bobwhites. Uninfected bobwhites were implanted with live eyeworms and monitored for 21 days to determine whether eyeworms could be transferred and to gather information about the life history of the eyeworm. Restraint and anesthesia procedures previously described were used just before eyeworm transfer. Once the eye was anesthetized, the upper eyelid was lifted to expose the eye,

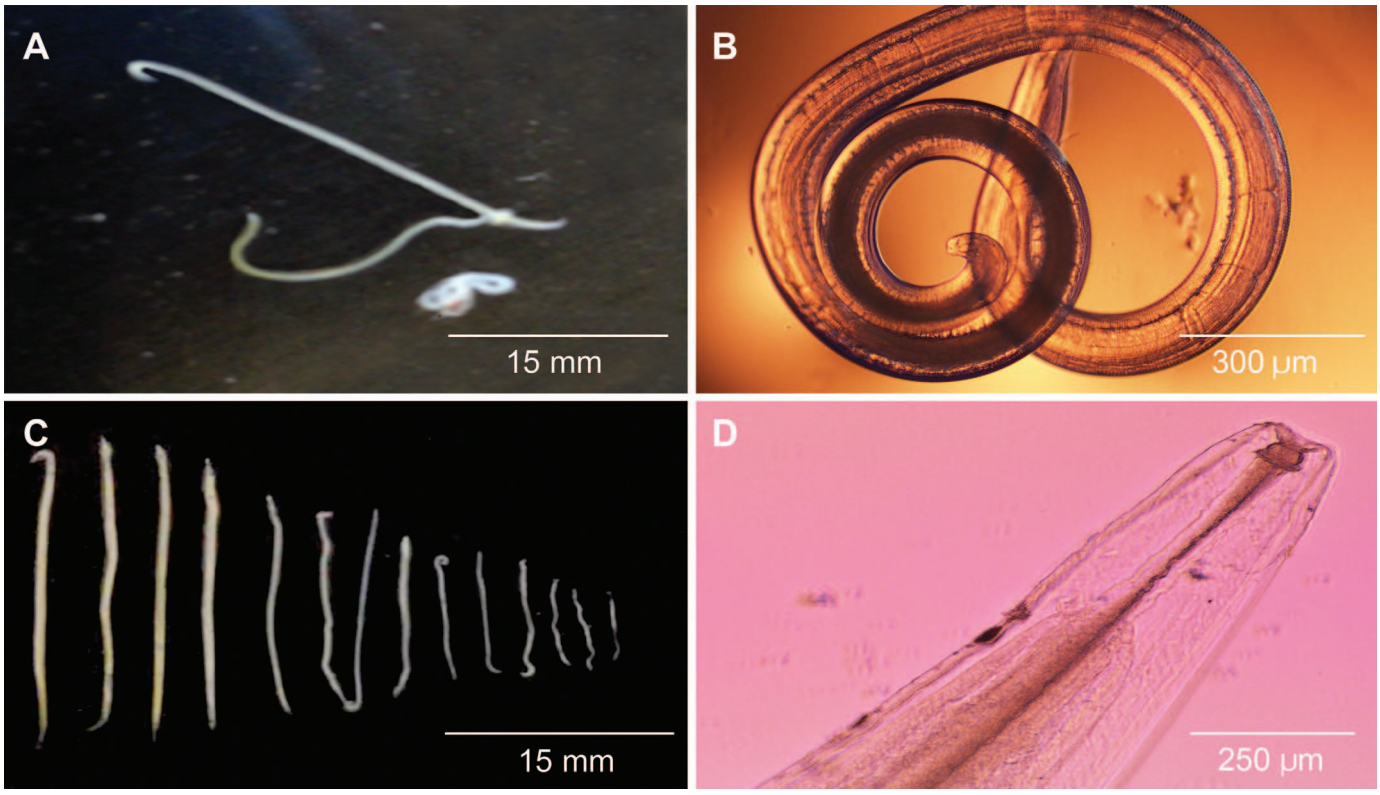

FIGURE 1. (**A**) Adult male and female *Oxyspirura petrowi* that migrated toward each other in the physiological saline solution and began mating for several minutes. (**B**) Adult male *O. petrowi* that recently ingested a blood meal from a northern bobwhite (*Colinus virginianus*). Dark portion in the esophagus is the ingested blood. (**C**) Juvenile and adult *O. petrowi* that were removed from northern bobwhites captured in Mitchell County, Texas, from June 2013 to September 2013. (**D**) Head structure of an adult female *O. petrowi*.

and eyes were infected with eyeworms. The infection level for each of 3 bobwhites per treatment group was 1 *O. petrowi* per eye. Infection levels were as follows for each of 2 bobwhites per treatment group: 3 *O. petrowi* per eye, 6 *O. petrowi* per eye, and 6 *O. petrowi* placed in only 1 eye. Juvenile *O. petrowi* were identified and placed in the bobwhites that had only 1 worm in each eye. The remaining birds had both juvenile and adult *O. petrowi*. Bobwhites were monitored twice a day (morning and night) for signs of eye irritation, redness, inflammation, and excessive scratching. In addition, 2 drops of fluorescein eye stain were applied to each of bobwhite's eyes to determine whether the transfer procedure produced any lacerations or lesions to the eyelid, eye surface, and nictitating membrane. Tissues where *O. petrowi* were found were macroscopically examined for damage caused by infection.

During the extraction process, *O. petrowi* were observed evading forceps. Evading was also seen during the extraction process after bobwhites were euthanized. Eyeworms were observed evading and moving from eye to eye via the nasal cavity and its associated ducts. With the bobwhite beak removed and nasal cavity fully exposed during removal, eyeworms were seen moving from the eye that was being examined to the other eye. When we switched to examine the other eye, eyeworms were seen moving back to the other eye via the nasal cavity. This behavior was observed until all of the eyeworms were extracted.

TABLE I. Results of 21-day experimental transfer of eyeworms (*Oxyspirura petrowi*) from wild-captive adult northern bobwhites (*Colinus virginianus*) captured in Mitchell County, Texas, to uninfected pen-raised adult northern bobwhites during June 2013.

Infection type	No. of bobwhites per treatment	No. of eyeworms transferred	No. of eyeworms recovered
1 eyeworm per eye	3	6	2
3 eyeworms per eye	2	12	2
6 eyeworms per eye	2	24	7
6 eyeworms in 1 eye	2	12	3

* Note: Eyeworms were recovered in both eyes, despite being transferred into only one eye.

Excised *O. petrowi* that were placed in room temperature physiological saline were noticeably sluggish and slow moving. However, when transferred to a solution closer to live bird temperature (37 C), worms began to become more active and oscillate back and forth. This heightened level of activity was also observed when *O. petrowi* were exposed to a light source. When *O. petrowi* were initially placed into the 10% saline solution held at 37 C, males and females were observed migrating across the petri dish toward each other, attaching for several minutes, and exhibiting mating behavior (Fig. 1A). Mating behavior was observed multiple times on different occasions within the same petri dish.

Gravid female egg development was documented throughout the experiment. Eggs were densely packed within the body. The anterior portion of gravid females had primarily undeveloped eggs, but posteriorly the eggs appeared to be more developed and were excreted out the vaginal pore.

When a blood meal was introduced into the saline holding solution, several *O. petrowi* quickly attaching themselves to the blood clot and/or piece of bobwhite tissue, whereas the rest of their body actively oscillated back and forth. They remained attached for several hours despite being prodded with forceps. Also, microscopic observation of a few recently collected *O. petrowi* from necropsied bobwhites revealed a red-brown coloration within the esophagus, suggesting blood ingestion (Fig. 1B).

Results of the live *O. petrowi* transfers to pen-raised bobwhites are presented in Table I. When the transferred eyeworms were placed into the treatment bobwhite's eyes, they quickly self-orientated and moved under

TABLE II. Eyeworm (*Oxyspirura petrowi*) survival experiment using 10 types of liquid media. Eyeworms cultured in media 1–7 were maintained in petri dishes at 40 C for 35 days. Eyeworms cultured in media 8–11 were incubated in 6-well culture plates in an Isotemp CO_2 incubator maintained at 32 C with a constant 5% CO_2 intake.

Medium	No. live worms	Day 1	Day 2	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 35
1. 10% saline solution	4	4	1	0	0	0	0	0	0	0
2. 10% saline solution with bobwhite blood/tissue	4	4	3	0	0	0	0	0	0	0
3. Equate artificial tears	4	4	0	0	0	0	0	0	0	0
4. Equate artificial tears with bobwhite blood/tissue	4	4	1	0	0	0	0	0	0	0
5. BioTrue artificial tears	4	4	1	0	0	0	0	0	0	0
6. BioTrue artificial tears with bobwhite blood/tissue	4	4	1	0	0	0	0	0	0	0
7. 10% sucrose solution	24	24	10	0	0	0	0	0	0	0
8. Phosphate buffered saline solution	24	24	24	5	0	0	0	0	0	0
9. Physiological saline recipe solution	24	24	24	4	0	0	0	0	0	0
10. 5 ml of physiological saline recipe solution with 5 ml of egg whites	24	24	24	20	10	4	0	0	0	0
11. 5 ml of physiological saline recipe solution with 5 ml of egg whites cultured under sterile conditions	48	48	48	48	36	23	11	6	3	3

* Note: Four eye worms were placed in each of the 6-well culture plates in media 8–10 and 8 per well in media 11.

the eyelid or nictitating membrane out of sight. Of the 54 eyeworms placed into uninfected pen-raised bobwhites during the experimental transfer, 26% of them were recovered 21 days later. By the end of the 21-day exposure period, juvenile *O. petrowi* were noticeably larger and appeared to be adults. Collection of *O. petrowi* showed distinct differences in size as well as coloration throughout the duration of the field trapping activities (Fig. 1C).

One hundred and sixty-eight live *O. petrowi* were cultured in vitro and tested to determine their ability to survive outside of the host for long periods. The first 7 types of liquid culture media were placed in standard petri dishes (VWR 25384-062) with covers and maintained on a 40 C hot plate: 10% saline solution, 10% saline solution with bobwhite blood/tissue, Equate artificial tears (Equate Brands), equate artificial tears with bobwhite blood/tissue, BioTrue artificial tears (Bausch & Lomb, Rochester, New York), BioTrue artificial tears with bobwhite blood/tissue, and 10% sucrose solution. An additional 3 liquid culture media were tested to determine their ability to maintain live *O. petrowi*: physiological saline recipe (Corba et al., 1969), phosphate buffered saline, and egg white with physiological saline solution. Each was placed in a multiwell™ 6-well culture plate (Falcon 35–3224) and maintained at 32 C with a 5% CO_2 intake in an Isotemp CO_2 incubator (model 3550; Fisher Scientific, Pittsburgh, Pennsylvania). Four live *O. petrowi* were placed in each of the media culture plates or media plates and monitored twice daily until all worms were dead. Each worm was examined every 12 hr for the signs of movement using a magnifying ocular headset and a light until all eyeworms were dead. Several weeks after death, a gravid female decomposed sufficiently for the eggs to be released, and the eggs dispersed throughout the well. The well plate was then placed back inside the incubator because *O. petrowi* in the 5 remaining wells were still alive. The physiological saline recipe and egg white mixture was later repeated using 8 live *O. petrowi* per 6-well culture plate.

The survival experiment results using the 10 liquid media are presented in Table II. *Oxyspirura petrowi* placed in egg white and physiological saline recipe were sustained and kept alive for approximately 17 days. The same media were then replicated again, and *O. petrowi* were sustained for 36 days, with 23 of 24 surviving to 15 days, compared with those in other solutions that died within 48 hr (Table II). All other *O. petrowi* survived no more than 5 days in their respective media (Table II).

There was no macroscopic evidence of eye surface or nictitating membrane damage associated with *O. petrowi* infections. However, upon examination of the lacrimal duct, visible inflammation and petechial hemorrhaging were observed. In addition, upon necropsy, hemorrhaging was noted in the sinus mucosal tissues when *O. petrowi* were present. Examination of the head of *O. petrowi* suggests that this nematode has the necessary structures for tissue attachment and lysis of sinus tissues to feed on blood (Fig. 1D). *Oxyspirura petrowi* were also observed with bobwhite tissue still attached upon being excised from the quail.

Most studies on *O. petrowi* have found 40–60% prevalence and 6–15 worms per infected host (Jackson, 1969; Robel et al., 2005; Villarreal et al., 2012), which is problematic for researchers wishing to conduct pen studies requiring sufficient numbers of worms to examine intensity-dependent influences on infected hosts. Sustained maintenance of *O. petrowi* in egg white and physiological saline media allows researchers to "bank" live worms extracted from infected, wild-captured hosts for 5–15 days without substantial worm mortality. It is likely that increased *O. petrowi* mortality after 15 days in the egg white and physiological saline media resulted from depletion of the nutrients within the culture plate and needs further study to see whether *O. petrowi* can be successfully maintained in vitro for longer periods.

We also demonstrated that *O. petrowi* can successfully be removed from infected bobwhites without harming either live hosts or eyeworms. In addition, both juvenile and adult *O. petrowi* individuals were successfully transferred to uninfected bobwhites. These extraction and transplantation procedures allow for the use of live *O. petrowi* in laboratory studies examining life history and facilitates pen studies of captive quail examining potential negative impacts of *O. petrowi* on their hosts.

Additional insight regarding *O. petrowi* life history was documented throughout the course of this study. *Oxyspirura petrowi* was observed feeding on host blood and tissues associated with the sinuses, suggesting that high protein food resources are used instead of host tears. Searching of the nasolacrimal duct for *O. petrowi* has been previously reported for wild birds (Robel et al., 2003, 2005), but references to pathological responses to infection are lacking. The present study represents the first report of inflammation and petechial hemorrhaging of the nasolacrimal duct in association with *O. petrowi* infections.

Based on our findings of *O. petrowi* in the nasolacrimal duct, nasal sinuses, and eye surface under the nictitating membrane, infection could result in multiple impacts to infected hosts, including impaired respiratory capacity, visual obstruction, hemorrhage, increased energy expenditures, and increased susceptibility to secondary infections. Consequently, negative impacts of *O. petrowi* may be more extensive than previously believed. In addition, findings underscore the importance of examination for *O. petrowi* in multiple locations within the host for better assessment of prevalence and intensity of infection. Given the interest in learning more about *O. petrowi* in wild quail, our study provides the framework for researchers wishing to conduct in-depth investigations within a laboratory or aviary setting to elucidate life history attributes of *O. petrowi* and to assess the consequences of infections within their hosts.

Funding was provided by the Rolling Plains Quail Research Foundation. We thank Dr. Ernest Smith and Jibao He for microscopy and picture help and Steve White and Randy Bullard for property access.

LITERATURE CITED

Corba, J., B. Scales, and G. Froyd. 1969. The effect of DL-Tetramisole on *Thelazia rhodesii* (eyeworm) in cattle. Tropical Animal Health and Production **1:** 19–22.

Fielding, J. W. 1926. Preliminary note on the transmission of the eyeworm of Australian poultry. Australian Journal of Experimental Biology and Medical Science **3:** 225–232.

Jackson, A. S. 1969. Quail management handbook for west Texas Rolling Plains. Parks and Wildlife Department, Austin, Texas, Bulletin No. 48, 75 p.

McClure, H. E. 1949. The eyeworm, *Oxyspirura petrowi*, in Nebraska pheasants. Journal of Wildlife Management **13:** 304–307.

Pence, D. B. 1972. The genus *Oxyspirura* (Nematoda: Thelaziidae) from birds in Louisiana. Proceedings of the Helminthological Society of Washington **39:** 181–183.

Ransom, B. H. 1904. Manson's eye worm of chickens (*Oxyspirura mansoni*), with a general review of nematodes parasitic in the eyes of birds, and notes on the spiny-suckered tapeworms of chickens (*Davinea echinobothrida* (=*Taenia botrioplites*) and *D. tetragona*). USDA Bureau of Animal Industry, Bulletin No. 60, 72 p.

Robel, R. J., T. L. Walker Jr., C. A. Hagen, R. K. Ridley, K. E. Kemp, and R. D. Applegate. 2003. Helminth parasites of lesser prairie-chicken *Tympanuchus pallidicinctus* in southwestern Kansas: Incidence, burdens and effects. Wildlife Biology **9:** 341–349.

———, ———, R. K. Ridley, K. E. Kemp, and R. D. Applegate. 2005. Helminth parasites in ring-necked pheasant from southwestern Kansas. Prairie Naturalist **37:** 144–150.

Ruff, M. D. 1984. Nematodes and acanthocephalans. *In* Diseases of poultry, 8th ed., M. S. Hofstad, B. W. Calnek, C. F. Helmboldt, W. M. Reid, and H. W. Yoder Jr. (eds.). Iowa State University Press, Ames, Iowa, p. 614–648.

———, and R. A. Norton. 1997. Nematodes and acanthocephalans. *In* Diseases of poultry, 10th ed., B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif (eds.). Iowa State University Press, Ames, Iowa, p. 815–850.

Sanders, D. A. 1928. Manson's eyeworm of poultry. Journal of the American Veterinary Association **25:** 568–584.

Schwabe, C. W. 1951. Studies on *Oxyspirura mansoni,* the tropical eyeworm of poultry. II. Life history. Pacific Science **5:** 18–34.

Villarreal, S. M., A. M. Fedynich, L. A. Brennan, and D. Rollins. 2012. Parasitic eyeworm *Oxyspirura petrowi* in northern bobwhites from the Rolling Plains of Texas, 2007–2011. Proceedings of the National Quail Symposium **7:** 241–243.

Xiang, L., F. Guo, H. Zhang, L. LaCoste, D. Rollins, A. Bruno, A. M. Fedynich, and G. Zhu. 2013. Gene discovery, evolutionary affinity and molecular detection of *Oxyspirura petrowi*, an eye worm parasite of game birds. BMC Microbiology **13:** 233.