Pathological Response of Northern Bobwhites to *Oxyspirura petrowi* Infections

Author(s): Andrea Bruno, Alan M. Fedynich, Autumn Smith-Herron, and Dale Rollins
Source: Journal of Parasitology, 101(3):364-368.
Published By: American Society of Parasitologists
DOI: http://dx.doi.org/10.1645/14-526.1
URL: http://www.bioone.org/doi/full/10.1645/14-526.1

J. Parasitol., 101(3), 2015, pp. 364–368

PATHOLOGICAL RESPONSE OF NORTHERN BOBWHITES TO *OXYSPIRURA PETROWI* INFECTIONS

Andrea Bruno, Alan M. Fedynich, Autumn Smith-Herron*, and Dale Rollins†

Caesar Kleberg Wildlife Research Institute, Texas A&M University–Kingsville, 700 University Boulevard, MSC 218, Kingsville, Texas 78363. Correspondence should be sent to: *andrea.bruno@students.tamuk.edu*

ABSTRACT: The effects of *Oxyspirura petrowi* infections in northern bobwhites (*Colinus virginianus*) are not well understood. While studies have reported *O. petrowi* infections, none has histopathologically examined the eye surface and intraorbital glands to assess cellular-level impacts associated with infection. This study is the first to document the histopathology associated with *O. petrowi* infections. *Oxyspirura petrowi* occurred on the eye surface as well as in the conjunctiva, lacrimal ducts, lacrimal glands, and Harderian glands. Histopathology showed infections of *O. petrowi* caused cellular damage to these tissues, scarring and interstitial keratitis of the cornea, and acinar atrophy of the Harderian gland.

Oxyspirura petrowi (Spirurida: Thelaziidae) Skrjabin, 1929 is a heteroxenous life cycle nematode reported in a number of galliform and passerine hosts. This nematode has a wide geographic range and appears to occur primarily in birds found in open grassland vegetation communities (Pence, 1972). This oxyspirurid is associated with the eyelid and nictitating membrane (Saunders, 1935; Cram, 1937; McClure, 1949), conjunctiva of the eyelids and nasolacrimal ducts (Hunter and Quay, 1953; Robel et al., 2003), orbital cavity (Barus, 1965; Addison and Anderson, 1969; Al-Moussawi and Mohammad, 2013), and nasal sinuses (Dunham et al., 2014).

Two species of oxyspirurids are known to occur in North American galliformes, *O. petrowi* and *Oxyspirura mansoni* (Pence, 1972). The former is found in wild birds and the latter in poultry. Because of the economic importance of the poultry industry, substantial efforts have been undertaken to understand the life cycle of *O. mansoni* and its associated pathogenicity (Ransom, 1904; Kobayashi, 1927; Sanders, 1929; Schwabe, 1950). Little is known about the life cycle of *O. petrowi*, but it is thought to be similar to *O. mansoni* (Wehr, 1972). In the few studies that report *O. petrowi* pathology in wild birds, McClure (1949) and Pence (1972) observed no gross damage to the eye, whereas Saunders (1935) observed ocular irritation. Although the above studies have provided some insight, it still remains unclear what impact, if any, *O. petrowi* has on wild hosts, particularly in regard to host response at the cellular and tissue levels.

The results from this study provide insight regarding *O. petrowi* individuals inhabiting the orbital cavity and eye surface and the effect this species has on the hosts' cellular tissue structure. Our study may pique the interest of other researchers studying helminths in wild avian hosts, thereby adding to our limited knowledge of *O. petrowi*. Additionally, this study provides researchers with the specific microhabitats where *O. petrowi* occurs, to ensure all individuals of this species are accounted for in helminth surveys.

Beginning in 2012, we began an assessment of *O. petrowi* in wild northern bobwhites (*Colinus virginianus*). This host species has recently received increased interest regarding parasitism and diseases as a potential contributing factor to population declines, with particular focus on eyeworms (Villarreal, 2012; Xiang et al., 2013; Dunham et al., 2014). The objectives of this study were to identify the specific microhabitats within the intraorbital system that *O. petrowi* individuals inhabit in northern bobwhites and to determine the pathological response of those tissues using histological methods.

MATERIALS AND METHODS

Study area

Northern bobwhites were collected from 7 private ranches in the southern Rolling Plains ecoregion of Texas. Much of this area is a mesquite short-grass savannah, and its topography is characterized as gently rolling. Annual rainfall in the region varies from 40 cm along the westward edge to 75 cm along the east (Rollins, 2007). The average temperatures are 15 to 17 C. A majority of the landscape has been converted to cropland (cotton and grain fields) or is used for cattle grazing.

Host and tissue collection

Hosts were collected in August and October of 2012 and 2013 using wire mesh funnel traps baited with grain sorghum and euthanized by cervical dislocation. In 2012, whole eyeballs were removed from the orbital cavity for assessment of infections under the nictitating membrane and damage to the eye surface. This sample (n = 10) consisted of 3 juvenile males, 3 juvenile females, 3 adult males, and 1 adult female. In 2013, whole heads were removed from each bird to assess pathological response of intraorbital gland tissue within the orbital cavity. This sample (n = 12) consisted of 4 juvenile males, 3 juvenile females, 2 adult males, and 3 adult females. Tissues were collected in both years from infected and non-infected northern bobwhites. Infection was determined post mortem by examination of the nictitating membrane and by examination of all ocular and accessory tissues in both collections. Tissues were fixed in 10% neutral buffered formalin.

To search for *O. petrowi* in the intraorbital glands of the 2013 sample, the upper and lower eyelids were removed via surgical scalpel to expose the entire nictitating membrane. The nictitating membrane was cut away, and the conjunctival sac was exposed. All adult worms were either removed from the sac and nasopharynx region or fixed within the tissues of the sac for histological examination. The lacrimal gland was located at the lateral canthus of the eye and removed. At the anterior lower edge of the conjunctival sac, the duct of the Harderian gland was located. The superior and inferior oblique muscles were severed, and the eye was removed. The branches of the external ophthalmic artery were severed at its points of entry into the inferior portion of the gland. Within the bony orbit, the rectus internus muscle was severed, and the Harderian gland removed. All extracted nematodes were post-fixed in acetic acid and stored in vials of 7% glycerol for positive identification and museum deposition. Voucher specimens were deposited in the Sam Houston State University Parasite Museum (SHSUP), Sam Houston State University, Huntsville, Texas. The voucher series consists of 16 vials containing juvenile, adult, male, and female specimens of *O. petrowi* in 1 lot (SHSUP 000,366 –

Received 25 March 2014; revised 11 February 2015; accepted 20 February 2015.

* Texas Invasive Species Institute; Sam Houston State University, P.O. Box 2506, Huntsville, Texas 77340.

† The Rolling Plains Quail Research Foundation, P.O. Box 61517, San Angelo, Texas 76906.

DOI: 10.1645/14-526.1

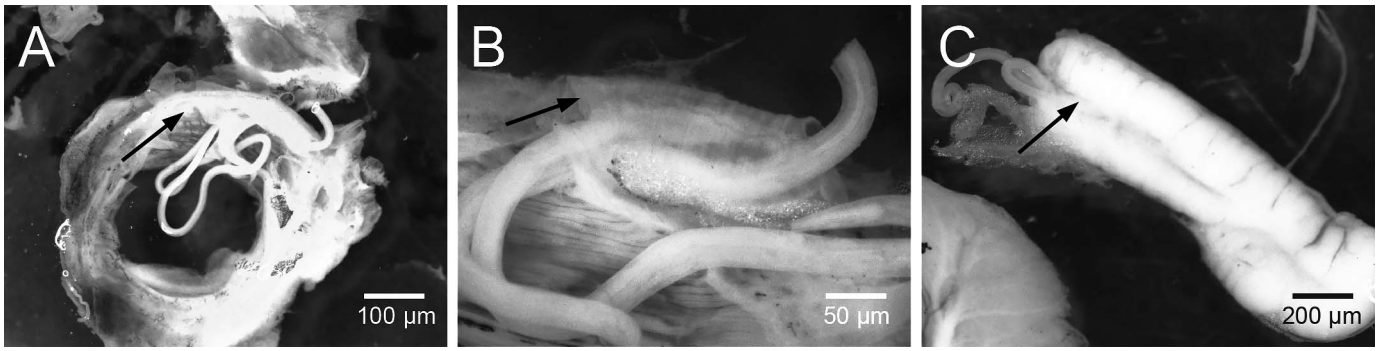

FIGURE 1. Specific microhabitats where *Oxyspirura petrowi* infection occurred in northern bobwhites (*Colinus virginianus*). (**A**) Conjunctiva (arrow) with adult *O. petrowi* individual residing in the inferior eye orbit. (**B**) The lacrimal duct (arrow) attached to the conjunctiva with adult *O. petrowi* individual emerging. (**C**) Harderian gland (arrow) with adult *O. petrowi* individual exiting through the central duct.

000,381) as follows: 131299, 130404, 130644, 130356, 131983, 131479, 131382, 130380, 131461, and 131431.

Northern bobwhites were collected in accordance with Texas Parks and Wildlife Department authorized scientific collection permit (SPR-0690-152), Texas A&M University Institutional Animal Care and Use Committee (AUP 2011-193), Texas A&M University–Kingsville (TAMUK) Institutional Animal Care and Use Committee (2009-09-21A), and TAMUK Institutional Biosafety Committee (IBC-ID No. 009-2011).

Histology and microtechniques

Glandular and ocular tissues were post-fixed in AFA overnight. Standard histological techniques were used (Humason, 1962). Tissues were sectioned at 7 µm thickness, and prepared sections were stained in hematoxylin and eosin (H&E).

Examination of prepared tissue sections was conducted using an Olympus B-Max 41 compound microscope (Olympus America Inc., Center Valley, Pennsylvania). Digital photographs of microscopic images were taken using an Olympus DP-12 digital camera (Olympus America Inc.) using the aforementioned microscope.

Gross examination of the nictitating membrane and intraocular glands were conducted and digital images were taken with an Olympus DP-12 digital camera mounted on an Olympus SZ16 (Olympus America Inc.) dissecting microscope. To create composite images with extended depth of field (DOF), multiple images were taken and stacked using Combine ZP image stacking software. As a result, scale bars associated with Figure 1 are applicable to tissue structures (top image) and cannot be used to measure *O. petrowi* occurring in the lower portions of the stacked images.

Parasite identification

The identification of *O. petrowi* was based on the re-description of the species by Pence (1972) using the principal morphological characters of lengths of the esophagus and spicules and distance of the vulva opening from the anterior end.

RESULTS

Oxyspirura petrowi extraction

From the 2012 host collection, where only the eyeball was examined, *O. petrowi* was found associated with the eye surface and nictitating membrane (range 1–61 from both microhabitats combined) in 9 of the 10 northern bobwhites. From the 2013 collection, 11 of 12 northern bobwhites were infected with *O. petrowi* (Harderian gland, range 1– 6; lacrimal gland, range 1–3), even in instances where the eye and nictitating membrane of 3 individuals showed no gross signs of infection. Nematodes were observed associated with and extracted from the nictitating membrane, eye surface, conjunctival sac, lacrimal ducts and gland, and the central and primary ducts of the Harderian glands (Fig. 1).

Eyeball tissue diagnosis

In 2 eyeballs from 1 adult northern bobwhite with no infection of *O. petrowi*, relatively healthy corneal tissue was presented by a layer of neutrophils lining the endothelium (Fig. 2A). The endothelium, however, should be 1 to 2 cell layers thick (Patt and Patt, 1969). In this case, neutrophils were aggregated beneath the endothelium. Microscopically, the corneal stroma and epithelium of the infected northern bobwhite eye displayed signs of connective tissue infiltration by inflammatory cells, vascularization, and epithelial distress (Fig. 2B). All northern bobwhite eyes that were collected from infected individuals exhibited interstitial (stromal) keratitis. This is a condition that is characterized by the aggregation/infiltration of a mixed population of inflammatory cells, including lymphocytes and macrophages. In addition, corneal scarring was evident by the presence of the irregular woven fibers (Fig. 2B).

Intraorbital gland tissue diagnosis

Histological sections of the lacrimal and Harderian glands demonstrated signs of pathology in individuals infected with *O. petrowi* (Figs. 3, 4). There were no apparent differences in the number of plasma cells associated with glands when comparing infected and non-infected northern bobwhites. However, there was an increase in the number of aggregated lymphocytes in the infected Harderian gland (Fig. 4). The most striking difference between non-infected and infected northern bobwhites was the degree of fibrosis with interlobular tissue spaces, and the progressive atrophy of acinar cells in infected hosts (Figs. 3, 4). In addition, there was noticeable displacement of the central duct of the Harderian gland as a result of *O. petrowi* infection. Gross pathology may result from the size of the nematode in comparison with the glands themselves or from the host's reaction to the parasite.

DISCUSSION

Prior to this study, the damage caused by *O. petrowi* was inconclusive. Saunders (1935) observed ocular irritation in parasitized sharp-tailed grouse (*Tympanuchus phasianellis*) and greater prairie-chickens (*Tympanuchus cupido*), but did not use histological techniques. McClure (1949) examined the eyes of infected ring-necked pheasants (*Phasianus colchicus*) and observed no apparent damage caused by *O. petrowi*. Pence (1972) found

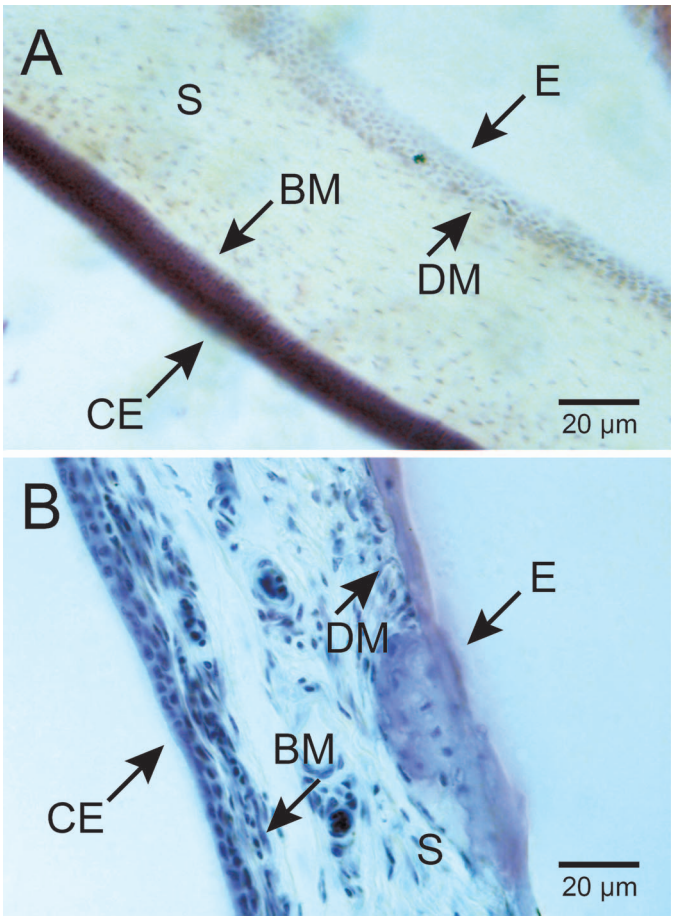

FIGURE 2. Histological section of a cornea from a non-infected and an *Oxyspirura petrowi* infected (n = 61) northern bobwhite (*Colinus virginianus*). (**A**) In non-infected tissue, the corneal epithelium (CE) is of appropriate thickness 5 to 7 cell layers thick; the stroma (S) appears healthy and consists of thin layers of fibers (opaque) with fibroblasts (fiber forming cells stained purple) embedded within. The endothelium (E), however, should be 1 to 2 cell layers thick. Neutrophils are aggregated beneath the endothelium, suggesting an infection within or around the anterior chamber of the eye. Hematoxylin and eosin (H&E). (**B**) In tissue infected with *O. petrowi*, a massive inflammatory reaction is apparent by the presence of immune cells within the corneal epithelium (CE), Descemet's membrane (DM), and within the connective tissue of the stroma (S). A thickening appearance is also apparent from scarring of the stroma (S). H&E. Abbreviation: BM = Bowman membrane.

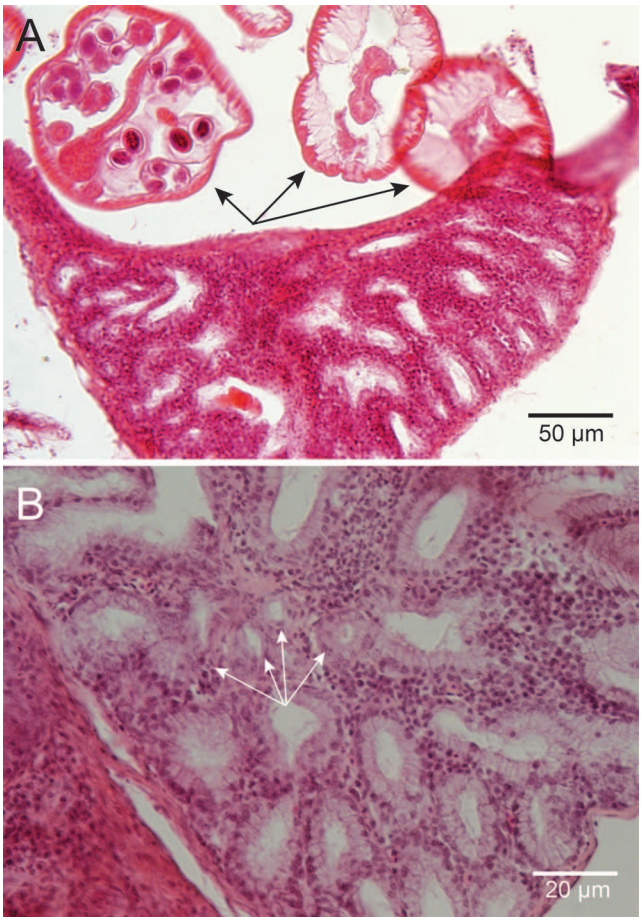

FIGURE 3. Histological sections of the lacrimal gland in a northern bobwhite (*Colinus virginianus*) infected with *Oxyspirura petrowi* (n = 4). (**A**) *Oxyspirura petrowi* (arrows) individual in cross section residing within the central duct of the lacrimal gland. Hematoxylin and eosin (H&E). (**B**) Lacrimal gland showing signs of acinar atrophy (arrows). H&E.

that *O. petrowi* infection caused no gross or pathologic changes even at high intensities (n = 30) in passerine birds.

Although little is known regarding pathological response of *O. petrowi* infections in wild birds, negative impacts have been reported in domesticated poultry infected with *O. mansoni* (Kobayashi, 1927; Sanders 1929; Schwabe, 1950; Wehr, 1972). Mechanical abrasion, mucosal discharge, and conjunctivitis have been reported in poultry infected with *O. mansoni* (see review in Ruff and Norton, 1997). Kobayashi (1927) also reported considerable damage to the conjunctiva and lacrimal ducts of chickens infected with *O. mansoni*. In a controlled experiment, Sanders (1929) found that the accumulation of decomposed *O. mansoni* coupled with inflammation and secondary infection eventually led to blindness in chickens with high intensities (n = 60) of infection.

Birds rely on their visual assessment of the environment to forage and evade predators (Jezler et al., 2010). The cornea is the most important structure of the ocular surface in terms of function (Knop and Knop, 2005). It is responsible for refracting light as well as allowing a sufficient quantity and quality of light to enter into the eye while enabling the retina to form an image (Jezler et al., 2010). Anatomically, the cornea is comprised of several layers: corneal epithelium, Bowman membrane, stroma, membrane of Descemet, and the endothelium (Bloom and Fawcett, 1994). During periods of inflammation, infection, or mechanical distress, lymphocytes penetrate and reside within the stroma. This inflammatory reaction was observed in the present study within the corneal epithelium, Descemet's membrane, and connective tissue of the stroma.

For regular maintenance (moistening, nutrition, and defense), the eye surface depends primarily on the conjunctiva and secretions from the lacrimal and Harderian glands, collectively called intraorbital glands (Knop and Knop, 2005). While the lacrimal gland is responsible for more aqueous secretions, the avian Harderian gland plays a vital role in controlling local orbital immunity (Dimitrov and Genchev, 2011; Kozlu and Altunay, 2011) via immunoglobulins IgA, IgG, and IgM, as well

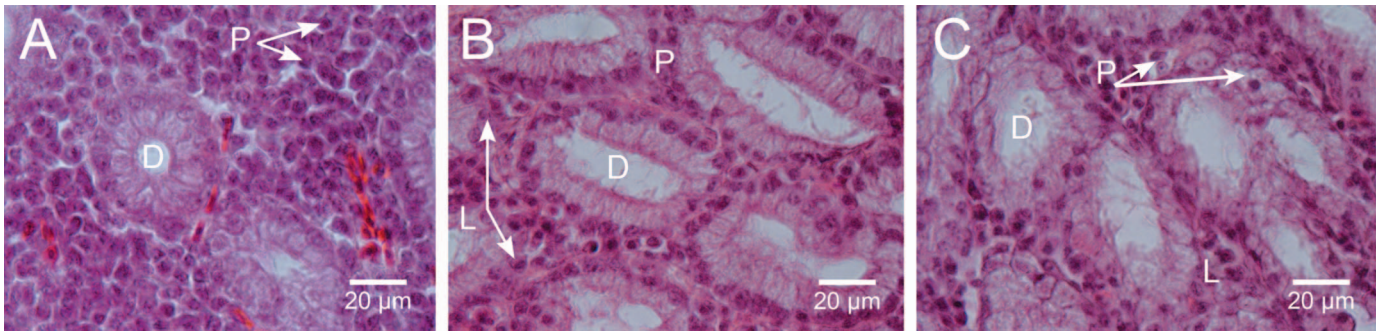

FIGURE 4. Histological sections of the non-infected and *Oxyspirura petrowi* infected (n = 6) Harderian gland in northern bobwhites (*Colinus virginianus*). (**A**) Cross section of a non-infected Harderian gland. Note the tubular secondary ducts (D) and plasma cells (P) within the interlobular connective tissue and presence of few lymphocytes. Hematoxylin and eosin (H&E). (**B**) Cross section of an infected Harderian gland with mild fibrosis and an increased presence of lymphocytes (L). H&E. (**C**) Cross section of an infected Harderian gland with acinar atrophy and the reduction of plasma cells (P). H&E.

as vascular released immune cells (macrophages, lymphocytes, granulocytes) onto the eye orbit (Baba et al., 1990; Boydak and Mehmet, 2009). In the present study, plasma cells (cells responsible for presenting antibodies) and other white blood cells were readily visible in histological preparations from both non-infected and infected Harderian glands. However, the number of plasma cells in infected hosts decreased, while the number of lymphocytes increased. In addition, a positive association occurred between heavily infected lacrimal and Harderian gland tissues and acinar atrophy. Acinar atrophy causes a decrease in secretory material and immune cells available to bathe and protect the outer eye surface. If a significant decrease in lubrication on the eye surface occurs, it could be particularly irritating to the host when *O. petrowi* also inhabits the nictitating membrane. A lack of aqueous, immune rich material on the eye surface where eyeworms are present could potentially contribute to corneal scarring and visual impairment.

General conclusions

Based on the present study using histopathological techniques, *O. petrowi* individuals found under the eyelid, nictitating membrane, and conjunctival sac are capable of causing corneal scarring, conjunctivitis, and keratitis (and associated inflammatory responses), and it is likely that mechanical abrasion and mucosal discharge are contributing factors in infections. In addition, *O. petrowi* individuals in the intraorbital glands elicit an inflammatory reaction, displace gland structure, and decrease secretory cells. The impact *O. petrowi* individuals have on the host's vision and function in the wild cannot be determined from the results of this study.

Oxyspirura petrowi has been reported in various species of wild birds (see reviews in Addison and Anderson, 1969; and Pence, 1972). However, it is often unclear whether researchers only examined the eye surface and nictitating membranes or all possible areas that *O. petrowi* inhabit. For example, studies on wild birds have specifically noted examining the orbital cavity (Barus, 1965; Addison and Anderson, 1969; Al-Moussawi and Mohammad, 2013), and lacrimal ducts (Robel et al., 2003) and nasal–lacrimal–orbital tissue (Dunham et al., 2014) for *O. petrowi* presence. The present study documents *O. petrowi* in the Harderian gland and lacrimal ducts in 8 of 11 (73%) northern bobwhites examined, of which 3 did not have eyeworms present on the eye surface or associated with the nictitating membrane. Helminth surveys in birds may underestimate eyeworm prevalence and intensity if the lacrimal gland, Harderian gland, and lacrimal ducts are not examined during necropsy.

Because adult *O. petrowi* individuals were observed in the Harderian gland, it is likely they are feeding on the glandular secretions. However, additional studies are needed to specifically determine what *O. petrowi* individuals are feeding on within each host organ in which they occur.

Our study is the first to document *O. petrowi* infection within the Harderian gland and eye pathology associated with *O. petrowi* using histological techniques. Based on these findings, additional studies are needed to determine whether *O. petrowi* infections could be a factor in decreasing survival in northern bobwhites.

ACKNOWLEDGMENTS

We thank the researchers and participants working under Operation Idiopathic Decline who assisted with the collection of the hosts examined in this study as well as research assistants Clayton Sublett and Melissa Sisson at the Texas Invasive Species Institute, Sam Houston State University. We also thank Dr. Mike Kinsella for his assistance in confirming identification of *Oxyspirura petrowi*. Texas A&M University–Kingsville does not endorse any of the commercial products used in this study. This research was funded by the Rolling Plains Quail Research Foundation and supported by the Caesar Kleberg Wildlife Research Institute and Sam Houston State University. This is manuscript No. 14-103 of the Caesar Kleberg Wildlife Research Institute.

LITERATURE CITED

ADDISON, E. M., AND R. C. ANDERSON. 1969. A review of eyeworms of the genus *Oxyspirura* (Nematoda: Spirurodidea). Journal of Wildlife Diseases **55:** 1–58.

AL-MOUSSAWI, A. A., AND M. K. MOHAMMAD. 2013. The eyeworm, *Oxyspirura petrowi* Skrjabin, 1929 (Nematoda, Thelaziidae) in the masked shrike *Lanius nubicus* Lichtenstein, 1823 (Passeriformes, Laniidae) collected in Baghdad City, Central Iraq. International Journal of Recent Scientific Research **4:** 1126–1128.

BABA, T., T. KAWATA, K. MASUMOTO, AND T. KAJIKAWA. 1990. Role of the Harderian gland in immunoglobulin: A production in chicken lacrimal fluid. Research in Veterinary Science **49:** 20–24.

BARUS, V. 1965. On some species of the genus *Oxyspirura* (Nematoda: Thelaziidae) from birds in Czechoslovakia. Journal of Helminthology **39:** 117–124.

BLOOM, R. E., AND D. W. FAWCETT. 1994. A textbook of histology, 12th ed. Chapman & Hall, New York, New York, 964 p.

Boydak, M., and F. A. Mehmet. 2009. Histology of the Harderian gland of domestic geese (*Anser anser domesticus*). Acta Veterinaria Brno **78:** 199–204.

Cram, E. B. 1937. A review of the genus *Oxyspirura*, with a morphological study of *O. petrowi* Skrjabin, 1929, recently discovered in galliform birds of the northern United States. *In* Papers on helminthology published in commemoration of the 30-year jubileum of K. I. Skrjabin and of 15th anniversary of the All-Union Institute of Helminthology. All-Union Lenin Academy of Agricultural Sciences, Moscow, Russia, p. 89–98.

Dimitrov, D. S., and A. G. Genchev. 2011. Comparative morphometric investigations of intraorbital glands in Japanese quails (*Coturnix coturnix japonica*). Bulgarian Journal of Veterinary Medicine **14:** 124–127.

Dunham, N. R., L. A. Soliz, A. M. Fedynich, D. Rollins, and R. J. Kendall. 2014. Evidence of an *Oxyspirura petrowi* epizootic in northern bobwhites (*Colinus virginianus*). Journal of Wildlife Diseases **50:** 552–558.

Humason, G. L. 1962. Animal tissue techniques, 2nd ed. W.H. Freeman & Company, San Francisco, 469 p.

Hunter, W. S., and T. L. Quay. 1953. An ecological study of the helminth fauna of Macgillivray's seaside sparrow, *Ammospiza maritime macgillivraii* (Audubon). American Midland Naturalist **50:** 407–413.

Jezler, P. C. O. C., M. B. P. Braga, E. Perlamann, R. Squarzoni, M. I. Borella, P. S. M. Barros, L. Milanelo, and A. Antunes. 2010. Histological analysis of eyeballs of the striped owl *Rhinoptynx clamator*. *In* Microscopy: Science, technology, applications and education, A. Mendez-Vilas and J. Diaz (eds.). Formatex, Badajoz, Spain, p. 1047–1054.

Knop, E., and N. Knop. 2005. The role of eye-associated lymphoid tissue in corneal immune protection. Journal of Anatomy **206:** 271–285.

Kobayashi, H. 1927. On the life history of *Oxyspirura mansoni* and pathological changes in the conjunctiva and the ductus lacrymalis caused by the worm. Japan Pathological Society Transactions **17:** 239–242.

Kozlu, T., and H. Altunay. 2011. Light and electron microscopic studies of the quail (*Coturnix coturnix*) Harderian gland. Journal of Animal and Veterinary Advances **10:** 932–938.

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

Patt, D. I., and G. R. Patt. 1969. Sense organs. *In* Comparative vertebrate histology. Harper and Row Inc., New York, p. 372–391.

Pence, D. B. 1972. The genus *Oxyspirura* (Nematoda: Thelaziidae) from birds in Louisiana. Proceedings of the Helminthological Society of Washington **39:** 23–28.

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. Journal of Wildlife Biology **9:** 341–349.

Rollins, D. 2007. Quail on the Rolling Plains. *In* Texas quails: Ecology and management, L. A. Brennan (ed.). Texas A&M University Press, College Station, Texas, p. 117–141.

Ruff, M. D., 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. 1929. Manson's eyeworm of poultry. Florida Agriculture Experimental Station Bulletin **206:** 567–584.

Saunders, G. B. 1935. Michigan's studies of sharp-tailed grouse. Transactions of the American Game Conference **21:** 342–344.

Schwabe, C. W. 1950. Studies on *Oxyspirura mansoni*, the tropical eyeworm of poultry. III. Preliminary observations on eyeworm pathogenicity. American Journal of Veterinary Research **11:** 286–290.

Villarreal, S. M. 2012. Helminth infections across the annual breeding cycle of northern bobwhites from Fisher County, Texas. M.S. Thesis. Texas A&M University–Kingsville, Kingsville, Texas, 82 p.

Wehr, E. E. 1972. Nematodes and acanthocephalans. *In* Diseases of poultry, M. S. Hofstad, B. W. Calneck, C. F. Helmboldt, W. M. Reid, and H. W. Yoder, Jr. (eds.). Iowa State University Press, Ames, Iowa, p. 844–883.

Xiang, L., F. Guo, H. Zhang, L. Lacoste, D. Rollins, A. Bruno, A. 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, doi:10.1186/1471-2180-13-233, p. 9.