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# HISTOPATHOLOGICAL ANALYSIS OF QUAILS IN THE TRANS-PECOS ECOREGION OF TEXAS

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## ABSTRACT

Quail populations in Texas, USA, have declined over the past few decades due primarily to habitat loss. The role that parasites may play in such declines has been a recent topic of concern. To help address this question, we collected 12 scaled quail (*Callipepla squamata*), 8 Gambel's quail (*Callipepla gambelii*), and 3 Montezuma quail (*Cyrtonyx montezumae*) from across the Trans-Pecos ecoregion of Texas via hunter harvest, funnel traps, and night netting. Quail samples were necropsied to determine the abundance of eyeworms (*Oxyspirura petrowi*). Histopathological analyses were conducted on quail eyeballs and periocular tissues to gain information on parasite-related tissue damage and document other pathogenic factors. We calculated mean abundances of *Oxyspirura petrowi* for sampled scaled ( $\bar{x} = 5.5$ , standard deviation [SD] = 2.5,  $\tilde{x} = 3$ ,  $n = 12$ ), Gambel's ( $\bar{x} = 6.4$ , SD = 4.2,  $\tilde{x} = 1.5$ ,  $n = 8$ ), and Montezuma quail ( $\bar{x} = 13$ , SD = 1.5,  $\tilde{x} = 13$ ,  $n = 3$ ). Host tissues exhibited immune responses (i.e., lymphocytic conjunctivitis and plasmacytic adenitis) to *O. petrowi*. The observed immune responses indicated relatively mild irritation within the ocular tissues. It has been speculated that such irritation to ocular tissues could negatively impact quail vision. This potential impact is worth noting because quails rely on keen vision to detect predators. Future research should focus on measuring the effects of *O. petrowi* infections on quail survival.

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**Key words:** *Callipepla gambelii*, *Callipepla squamata*, *Cyrtonyx montezumae*, Gambel's quail, histopathology, Montezuma quail, *Oxyspirura petrowi*, parasite, scaled quail, Texas

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Quail (family Odontophoridae) populations in Texas, USA, have experienced declines over the past several decades, primarily due to habitat loss and fragmentation (Brennan 2007, Hernández et al. 2012). Recently, it has been hypothesized that parasites, specifically the helminth *Oxyspirura petrowi*, may also influence declines (Dunham et al. 2014). *Oxyspirura petrowi* infect the periocular tissues of their definitive hosts (Bruno et al. 2015, Shea et al. 2020). The species is an indirect life cycle endoparasite transmitted to quail when they consume the intermediate host, insects (Kalyanasundaram et al. 2019). Most of the previous research efforts focused on effects that parasites may have on northern bobwhite (*Colinus virginianus*) in the Rolling Plains ecoregion of Texas and southern Texas (Olsen 2014, Bruno et al. 2015, Dunham et al. 2016b). Studies conducted in the Rolling Plains ecoregion documented pathological effects of *O. petrowi* on the ocular and periocular tissues of northern bobwhite (Bruno et al. 2015, Dunham et al. 2016b). Bruno et al. (2015) found signs of corneal scarring, keratitis, and conjunctivitis caused by *O. petrowi*. Furthermore, Dunham et al. (2016b) documented inflammation, fibrosis, and adenitis caused by *O. petrowi*.

While parasites of quail (primarily northern bobwhite) have been studied extensively in other parts of Texas, little research has been conducted on parasites infecting quails in Texas' Trans-Pecos ecoregion. Four species of quail inhabit the Trans-Pecos: scaled quail (*Callipepla squamata*), Gambel's quail (*Callipepla gambelii*), Montezuma quail (*Cyrtonyx*

*montezumae*), and northern bobwhite, though northern bobwhite are less common (Harveson 2007). Landgrebe et al. (2007) and Fedynich et al. (2019) surveyed portions of the Trans-Pecos ecoregion for quail endoparasites. However, both studies focused only on scaled quail and did not survey Gambel's or Montezuma quail. Due to differences in host life histories and habits, it is necessary to collect parasite data on all 3 species of quails (Harveson 2007). Additionally, only one of these studies (Bedford 2015) conducted histopathological analyses to determine whether parasites damaged the tissues that they infected. Histopathology, the study of changes in tissues caused by disease, is necessary to determine various pathogens' effects on their hosts (Stedman 1920, Cameron and Allen 2004). Staining cross-sections of tissues prior to viewing them under microscope, allows researchers to inspect tissue components at a cellular level (Cameron and Allen 2004, Gurcan et al. 2009). Past histopathological studies identified immune responses in the ocular tissues of quail due to *O. petrowi* infections (Bedford 2015, Bruno et al. 2015). Additionally, Bedford (2015) concluded that *O. petrowi* may be damaging their host tissues. Our objectives were 1) to determine whether scaled quail, Gambel's quail, and Montezuma quail were eliciting any immune responses to *O. petrowi*, 2) to determine whether there was a relationship between the level of elicited immune responses and parasite abundance, and 3) to determine whether *O. petrowi* were damaging the tissues that they infected in these quails.

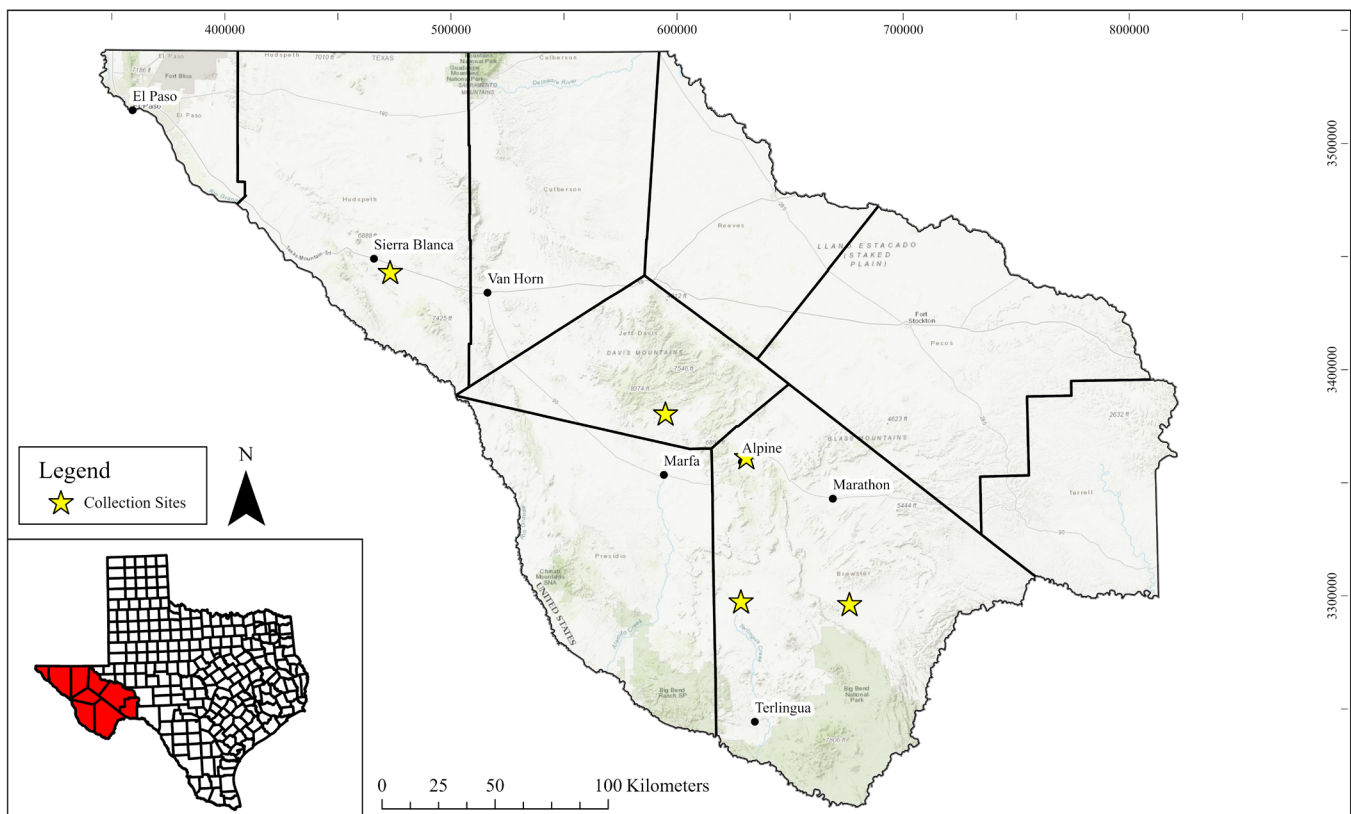


Fig. 1. Collection sites of desert quails that were used to determine the histopathological effects of *Oxyspirura petrowi*, April 2019–July 2020, in the Trans-Pecos ecoregion, Texas, USA.

## STUDY AREA

The Trans-Pecos ecoregion of Texas is the westernmost region of the state. It is west of the Pecos River and extends to the Texas-New Mexico state boundary line (Chapman and Bolen 2017). The temperature in the Trans-Pecos averages around 17.3° C (NOAA National Centers for Environmental Information 2019). The region receives as little as 20 cm of rainfall in the west and up to 50 cm in the east (Chapman and Bolen 2017). The elevation ranges from 762 m to 2,667 m (Harveson 2007). Soils are generally basic, consisting of deep sands and gravel mulch (Powell 1998). Chihuahuan Desert scrub, desert grassland, and pine (*Pinus* spp.)–oak (*Quercus* spp.)–juniper (*Juniperus* spp.) woodland vegetation types make up a large portion of the Trans-Pecos (Powell 1998). The flora of the region is diverse, consisting of 2,188 plant species, including creosote bush (*Larrea tridentata*), Mexican pinyon pine (*Pinus cembroides*), teddy-bear cholla (*Cylindropuntia bigelovii*), and buffalograss (*Bouteloua dactyloides*; Powell 1998, 2000).

## METHODS

### Sample Collection

We collected quail for histopathological analysis from 3 ranches in Brewster County and one in Hudspeth County (Figure 1). We collected quail opportunistically over 2 years via hunter harvest, walk-in funnel traps, and night netting. We collected quail outside of the hunting season under Texas Parks and Wildlife Department (TPWD) scientific permit number SPR-1118-296. Additionally, all research protocols were approved by the Sul Ross State University (SRSU) Institutional Animal Care and Use Committee (SRSU-IACUC 18-0006).

We used standard funnel traps (Stoddard 1931) to trap scaled quail at one collection site near Alpine, Texas, where hunting was not allowed. We placed 3 traps along ranch roads 3 hours before sunset, baited with milo (*Sorghum bicolor*), and checked at sunset. We euthanized quail captured in funnel-traps by cervical dislocation.

We conducted night netting with spotlights, dipnets, and bird dogs to collect scaled quail from a site where firearms were not allowed (Labisky 1968, Greene 2013). We used bird dogs to locate quail during the late afternoon before the night of captures. At night, bird dogs were used to get a general location of the quail, spotlights were used to determine the exact location of quail, and then dipnets were used to capture the quail. We euthanized quail trapped via spotlight and dip netting by cervical dislocation upon collection. We placed all quail into individual zipper bags post-harvest. We recorded collection site and date in the field. We assigned each quail an identification number associated with the collection site and date. We then transported collected quail to the laboratory in a small plastic cooler. We immediately necropsied all quail upon arrival at the laboratory.

### Sample Preparation

First, we determined age and sex of the quail. We aged scaled and Gambel's quail from 1 week to 12 weeks using primary feather replacement (Cain and Beasom 1983, Hernández and Guthery 2012). After 12 weeks, scaled and Gambel's quail were classified as a juvenile (J) or adult (A) by inspection of the primary covert feathers (Leopold 1939; Cain and Beasom 1983). We aged Montezuma quail by inspection of the greater primary coverts (Leopold 1939). We sexed all scaled, Gambel's, and Montezuma quail by examination of the gonads.

### Eyeworm Extractions

We examined quail for *Oxyspirura petrowi* using a methodology as described by Dunham et al. (2016a). We conducted examinations at this stage of the study without the aid of microscope because *O. petrowi* are macroscopic parasites. We lifted each eyelid using forceps to visually inspect for *O. petrowi*. Then, we removed eyelids with surgical scissors. The nictitating membrane was then lifted using a probe to examine for *O. petrowi*. After the initial examination, we removed the upper mandible, and made incisions along the nasal cavities using surgical scissors. Next, the eyeballs and periocular tissues, namely the Harderian gland, naso-lacrimal duct, and conjunctiva, were removed, placed into a petri dish, and examined for *O. petrowi* using curved forceps. At each step of the examination, any *O. petrowi* observed were extracted. Once extracted, we counted *O. petrowi* and preserved them in 10% neutral buffered formalin. We placed eyeballs and periocular tissues in 10% neutral buffered formalin for histopathological analysis.

## HISTOPATHOLOGICAL ANALYSES

We submitted fixed eyeballs and periocular tissues to the Texas A&M Veterinary Medicine Diagnostic Laboratory (TVMDL) in College Station, Texas, where Dr. Erin Edwards, a board-certified anatomic pathologist, conducted histopathological analyses. Formalin-fixed tissues were embedded in paraffin, sectioned at a thickness of 4 µm, and stained with modified hematoxylin and eosin using a Tissue-Tek Prisma Plus Automated Slide Stainer (Sakura Finetek USA, Inc., Torrance, CA, USA). Glass slides were digitally scanned with a Hamamatsu NanoZoomer S360 (Hamamatsu Photonics, Hamamatsu City, Shizuoka, Japan) and examined by Dr. Edwards. Immune responses identified within tissues were classified on an ordinal scale as either Minimal, Mild, Moderate, or Severe.

### Statistical Analyses

We used Spearman's correlation to determine whether there was a correlation between *O. petrowi* abundances and level of immune response. We used Spearman's correlation because the immune response variable was recorded as an ordinal variable (Laerd Statistics 2015). Spearman's correlation tested the strength and direction of the relationship between *O. petrowi* and immune response (Laerd Statistics 2015).

## RESULTS

We examined 12 scaled quail, 8 Gambel's quail, and 3 Montezuma quail for *O. petrowi*. Ten scaled quail, 5 Gambel's quail, and all 3 Montezuma quail were infected with *O. petrowi* (Table 1). We calculated mean abundances of *O. petrowi* for sampled scaled ( $\bar{x} = 5.5$ , standard deviation [SD] = 2.5,  $\tilde{x} = 3$ ,  $n = 12$ ), Gambel's ( $\bar{x} = 6.4$ , SD = 4.2,  $\tilde{x} = 1.5$ ,  $n = 8$ ), and Montezuma quail ( $\bar{x} = 13$ , SD = 1.5,  $\tilde{x} = 13$ ,  $n = 3$ ).

From histopathological analyses, quail eyes were diagnosed with plasmacytic adenitis and lymphocytic conjunctivitis within the periocular tissues. All individuals experienced multifocal levels of coalescing, chronic, plasmacytic adenitis within the Harderian gland attributed to *O. petrowi* infections (Table 2). Ten samples (4 scaled quail, 4 Gambel's quail, and 2 Montezuma quail) were also diagnosed with chronic lymphocytic conjunctivitis (Table 3). The Spearman's correlation concluded that there was no correlation between *O. petrowi* abundances and plasmacytic adenitis ( $r_s(23) = -0.200$ ,  $p = 0.360$ ). From the Spearman's correlation, we observed no correlation between *O. petrowi* abundances and lymphocytic conjunctivitis ( $r_s(10) = 0.116$ ,  $p = 0.597$ ).

Table 1. Abundances of *Oxyspirura petrowi* in 3 species of quail collected for histopathological analyses, April 2019–July 2020, in the Trans-Pecos ecoregion, Texas, USA.

Individual	Collection date	Quail species	Cohort <sup>a</sup>	<i>O. petrowi</i> abundance
1	Apr 2019	Scaled	AM	4
2	Apr 2019	Scaled	AF	2
3	Apr 2019	Scaled	JM	7
4	Apr 2019	Scaled	AF	8
5	Apr 2019	Scaled	JM	0
6	Apr 2019	Scaled	JF	3
7	Apr 2019	Scaled	AM	4
8	May 2019	Scaled	JF	3
9	Mar 2020	Scaled	AF	2
10	Mar 2020	Scaled	JM	32
11	Mar 2020	Scaled	JM	1
12	Apr 2019	Scaled	AM	0
13	May 2019	Gambel's	AM	35
14	May 2019	Gambel's	JM	9
15	May 2019	Gambel's	JM	0
16	Apr 2020	Gambel's	JM	0
17	Apr 2020	Gambel's	JF	2
18	Jun 2020	Gambel's	JF	4
19	Jun 2020	Gambel's	JM	0
20	Jun 2020	Gambel's	JM	1
21	May 2020	Montezuma	AM	15
22	Jul 2020	Montezuma	AF	11
23	Jul 2020	Montezuma	JM	13

<sup>a</sup> A = adult, J = juvenile, M = male, and F = female.

## DISCUSSION

Lymphocytic conjunctivitis and plasmacytic adenitis were common diagnoses among our samples. Lymphocytic conjunctivitis is a condition described as an inflammation of the conjunctiva, a thin clear tissue that lies over the white part of the eye and the eyelid, with lymphocytes (Sallinger 2010). Lymphocytes are a type of white blood cell that helps the body determine the appropriate immune response to foreign bodies (Stedman 1920). The other common diagnosis, plasmacytic adenitis, is a condition described as the inflammation of a gland with plasma cells (Stedman 1920, McMillan and Engelbert 1963). In this case, inflammation occurred in the Harderian gland (Peters 2004). Plasma cells are a type of white blood cell that produces antibodies which attack foreign bodies (Stedman 1920). The conjunctiva and Harderian gland are organs that *O. petrowi* infect and therefore it is likely the immune responses were associated with *O. petrowi* infection (Bruno et al. 2015, E. Edwards, TVMDL, personal communication).

One purpose of these histopathological analyses was to determine whether desert quail elicit an immune response to parasite infection. From these analyses, we concluded that all infected samples exhibited some degree of immune response to *O. petrowi*. However, there was no correlation between the level of immune response and *O. petrowi* infection. This may be due, in part, to the small sample size used for the analysis (Israel 1992). As is the case with most studies of wild populations, we were unable to control for potentially influential variables.

For instance, each sampled quail experienced a unique suite of stressors and available resources prior to collection. Stress and nutrient deficiency in avian species can result in decreased immunocompetence, the capacity of an individual to elicit an immune response (Latshaw 1991, Svensson et al. 1998). Immunosuppressed individuals are more prone to parasitic infections (Evering and Weiss 2006). Individual #10 was infected with 35 *O. petrowi* and was diagnosed with minimal plasmacytic adenitis (Table 2). A lower level of immune response and a higher level of parasitic infection could mean that the individual had a dampened immune system before infection. Conversely, Individual #5 did not have any *O. petrowi* present but was diagnosed with severe plasmacytic adenitis (Table 2). The plasma cells, present in greater numbers in the Harderian gland, may be remnants from a previous infection.

It is possible that the immune responses observed during histopathological analyses were residual effects from previous parasitic infections. The duration of an infection may influence the level of immune response exhibited. This may also explain why immune responses were not observed in parasitized individuals. If the hosts were infected recently, it is possible that the body had not exhibited an immune response yet. A study conducted on pen-raised quail in a controlled environment is necessary to isolate the variable of interest.

These histopathological analyses were also used to determine whether *O. petrowi* were damaging tissues. Similar to previous studies, our sample size was small,

Table 2. *Oxyspirura petrowi* abundances and plasmacytic adenitis severity in quails collected, April 2019–July 2020, in the Trans-Pecos ecoregion, Texas, USA.

Individual	Collection date	Quail Species	Cohort <sup>a</sup>	<i>O. petrowi</i> abundance	Plasmacytic adenitis <sup>b</sup>
1	Apr 2019	Scaled	AM	4	Mild
2	Apr 2019	Scaled	AF	2	Mild
3	Apr 2019	Scaled	JM	7	Moderate
4	Apr 2019	Scaled	AF	8	Mild
5	Apr 2019	Scaled	JM	0	Severe
6	Apr 2019	Scaled	JF	3	Mild
7	Apr 2019	Scaled	AM	4	Moderate
8	May 2019	Scaled	JF	3	Moderate
9	Mar 2020	Scaled	AF	2	Moderate
10	Mar 2020	Scaled	JM	32	Moderate
11	Mar 2020	Scaled	JM	1	Moderate
12	Apr 2019	Scaled	AM	0	Moderate
13	May 2019	Gambel's	AM	35	Minimal
14	May 2019	Gambel's	JM	9	Moderate
15	May 2019	Gambel's	JM	0	Mild
16	Apr 2020	Gambel's	JM	0	Mild
17	Apr 2020	Gambel's	JF	2	Mild
18	Jun 2020	Gambel's	JF	4	Minimal
19	Jun 2020	Gambel's	JM	0	Mild
20	Jun 2020	Gambel's	JM	1	Mild
21	May 2020	Montezuma	AM	15	Mild
22	Jul 2020	Montezuma	AF	11	Mild
23	Jul 2020	Montezuma	JM	13	Mild

<sup>a</sup> A = adult, J = juvenile, M = male, and F = female.

<sup>b</sup> Categories are based on Texas A&M Veterinary Medicine Diagnostic Laboratory methodology.

Table 3. *Oxyspirura petrowi* abundances and lymphocytic conjunctivitis severity in quails collected, April 2019–July 2020, in the Trans-Pecos ecoregion, Texas, USA.

Individual	Collection date	Quail species	Cohort <sup>a</sup>	<i>O. petrowi</i> abundance	Lymphocytic conjunctivitis <sup>b</sup>
2	Apr 2019	Scaled	AF	2	Minimal
4	Apr 2019	Scaled	AF	8	Mild
5	Apr 2019	Scaled	JM	0	Mild
7	Apr 2019	Scaled	AM	4	Minimal
14	May 2019	Gambel's	JM	9	Moderate
15	May 2019	Gambel's	JM	0	Minimal
17	Apr 2020	Gambel's	JF	2	Mild
18	Jun 2020	Gambel's	JF	4	Mild
22	Jul 2020	Montezuma	AF	11	Mild
23	Jul 2020	Montezuma	JM	13	Minimal

<sup>a</sup> A = adult, J = juvenile, M = male, and F = female.

<sup>b</sup> Categories are based on Texas A&M Veterinary Medicine Diagnostic Laboratory methodology.

and inferences should be made cautiously (Bedford 2015, Bruno et al. 2015, Dunham et al. 2016b). Histopathological analyses in northern bobwhite collected from the Rolling Plains ecoregion found signs of keratitis, conjunctivitis, and adenitis caused by *O. petrowi* (Bruno et al. 2015, Dunham et al. 2016b). Inflammatory responses were noted in scaled quail collected in Texas' Rolling Plains ecoregion (Fedynich et al. 2019). Similarly, we documented lymphocytic conjunctivitis and plasmacytic adenitis in a large portion of our samples. Northern bobwhite in the Rolling Plains were also diagnosed with corneal scarring, corneal ulcerative erosions, and lesions within the Harderian gland (Bruno et al. 2015, Dunham et al. 2016b). Bedford (2015) concluded that *O. petrowi* could cause pathological damage to host tissues. In contrast, we found no evidence to suggest that *O. petrowi* damaged host tissues that they infected. The observed immune responses, lymphocytic conjunctivitis, and plasmacytic adenitis do not indicate severe tissue damage but rather a tissue irritation (E. Edwards, TVMDL, personal communication). Additionally, there were no signs of lesions, ulceration, or necrotic tissue commonly found when tissue damage occurs (E. Edwards, TVMDL, personal communication). Instead, our results indicate that only tissue irritation was occurring due to parasitic infection.

## MANAGEMENT IMPLICATIONS

We confirmed the presence of quail endoparasites in scaled, Gambel's, and Montezuma quail in the Trans-Pecos ecoregion of Texas and suggest that quail biologists and managers should discuss potential management strategies. The immune responses in quail detected from the histopathological analyses were indicative of tissue irritation and not damage. Moreover, we were unable to relate these immune responses directly to parasitic infection. A study conducted in a controlled, artificial environment would be useful for answering this question. However, without knowing how *O. petrowi* affects quail survival, it will be difficult to determine what, if any, science-based management actions should be taken.

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