DOI: 10.7589/JWD-D-23-00102
Journal of Wildlife Diseases, 00(00), 2024, pp. 000–000

Surveillance for Selected Pathogens and Parasites of Northern Bobwhite (*Colinus virginianus*) from Western Oklahoma, USA, 2018–20

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ABSTRACT: The Northern Bobwhite (*Colinus virginianus*) has been undergoing a range-wide population decline. Potential causes for declines across its historic range have been investigated for decades and include habitat loss and fragmentation and a variety of parasitic and infectious diseases. Although there have been studies on bobwhite ecology in Oklahoma, USA, relatively little is known about parasites and pathogens in the region. We evaluated the health of free-ranging bobwhites from nine sites in western Oklahoma. From 2018 to 2020, 206 bobwhites were evaluated for gross and microscopic lesions and tested for selected pathogens. In general, bobwhites were in good nutritional condition with ample muscle mass and fat stores. No significant gross lesions were observed in any bobwhite and no significant histologic lesions were detected in a subset. There was no evidence of infection with or exposure to reticuloendotheliosis virus, West Nile virus, respiratory Mycoplasmataceae species, *Pasteurella multocida*, intestinal *Eimeria* spp., or oral *Trichomonas* spp. Several pathogens of potential concern were detected, including avian adenovirus (8.6%), *Toxoplasma gondii* (2.3%), and haemosporidians (a *Haemoproteus* sp. (1.5%), *Leucocytozoon schoutedeni* (1.5%), and *Plasmodium homopolare* haplotype 2 [lineage LAIRI01; 3.6%]). *Physaloptera* sp. (12%) and *Sarcocystis* sp. (1%) were detected in the breast muscle. Low intraspecific genetic diversity was noted for *Physaloptera* sp., and sequences were most similar to *Physaloptera* sequences from bobwhites and grasshoppers (Orthoptera) in Texas. Low intensities of chewing lice, chiggers, and ticks were observed. A subset of bobwhites had evidence of exposure to selected toxicants and heavy metals; a small number had low levels of iron, manganese, zinc, molybdenum, and copper, which were not considered diagnostically relevant. In general, bobwhites from western Oklahoma appeared to be in good health with a low diversity of pathogens detected, but future work is needed to understand potentially changing disease risks for this population.
Key words: Galliformes, population decline, parasites, pathogens, toxins.

INTRODUCTION

The Northern Bobwhite (hereafter, bobwhite; *Colinus virginianus*), is one of the most popular game species, and it has high economic importance (Kellogg and Calpin 1971). Since the mid-1960s, the bobwhite population has undergone an approximately 85% decline throughout its historic range (about 4% decrease/year). In most regions density has decreased, with complete disappearance apparent in some areas (Sauer et al. 2017). Currently, Oklahoma, US, has one of the most robust populations of bobwhite, resulting in considerable interest among hunters; however, even these populations have undergone recent declines. The North American Bird Breeding Survey has provided population data on over 425 species of birds, including the bobwhite, for over 50 yr using volunteer roadside surveys and rigorous protocols, enabling the tracking of long-term population trends (Sauer et al. 2013, 2017). In Oklahoma, statewide roadside surveys are frequently employed to count bobwhites from vehicles along 20-mile-long routes (Janus 2018; Judkins 2020). This population survey method is conducted twice a year in Oklahoma, and statewide roadside survey averages have fallen from approximately 11 bobwhites observed per 20-mile route in 1990 to approximately 1.5 in 2020, with a nearly 42% drop between 2019 and 2020 (Judkins 2020). The 2020 roadside quail survey results were also 68% below the 31-yr (1990–2020) average of 5.1 (Janus 2018; Judkins 2020). Other population monitoring methods such as spring whistle calling, fall covey flushing, and numbers of reported hunter-harvested bobwhites reflect the same trend of decreasing bobwhite populations in Oklahoma (Judkins 2020). Thus, although the bobwhite population in Oklahoma is faring better than populations in other parts of their endemic range, it is experiencing the same overall population decline trend (Hernández et al. 2013).

Potential causes for the significant population decline of bobwhites across their historic range have been investigated for decades. Effects of habitat loss and fragmentation on local populations have been observed, with increases in roadways, croplands, and ranchland being inversely correlated with the observed numbers of bobwhites (Brennan and Kuvlesky 2005; Doggett and Locher 2018; Miller et al. 2019). Farming not only changes the habitat and increases fragmentation, possibly making it less favorable for foraging or nesting success or predator avoidance, but also may cause potential exposures to toxicants (e.g., pesticides), which can result in negative effects (Palmer and Bromley 1992; Richardson et al. 2020). In addition, many historical studies on parasites and pathogens have shown that pathogens may be a health threat (King et al. 1981; Wilson and Crawford 1988; Turaga et al. 2016; Dunham et al. 2017; Brym et al. 2018; Bruno et al. 2019; Shea et al. 2021). However, contemporary studies on free-ranging bobwhite quail have primarily focused on parasites or specific infectious agents (e.g., influenza virus, West Nile virus [WNV], intestinal microbiota) or were limited in geographic scope (Ferro et al. 2012; Urban et al. 2013; Su et al. 2014).

In Oklahoma, there have been many studies on the ecology of bobwhites and possible threats to populations (e.g., habitat, movements, and predation), but there are few contemporary data on bobwhite health. We aimed to establish baseline health data on bobwhites across western Oklahoma by conducting gross and microscopic postmortem examinations and laboratory testing for selected pathogens and toxicants that are known or suspected causes of morbidity and mortality.

MATERIALS AND METHODS

Study sites, animal capture, handling, and sampling

From 2018 to 2020, bobwhites were sampled from nine Wildlife Management Areas (WMAs) in western Oklahoma as described (Fig. 1; Wyckoff et al. 2023). Briefly, each year, 60×50×20 cm (24×24×8 in) funnel wire traps were deployed for two 2-wk sessions in early August and mid-October, for a total of six trapping sessions. Bobwhites were weighed in a bag on a Pesola spring scale (Schindellegi, Switzerland). For a subset of bobwhites, a blood sample was collected from the jugular vein using a 29-gauge, 1-mL insulin syringe (VetOne, Boise, Idaho, USA); part was deposited into a serum separator tube (Microtainer®, Franklin Lakes, New Jersey, USA), and the remaining was applied to a Nobuto filter strip (Toyo Roshi Kaisha, Ltd., Tokyo, Japan; Advantec MFS Inc., Dublin, California, USA distributor). The tubes were centrifuged at 18,000 × G (13,500 rpm) for 2 min and serum frozen at −20 C until testing. Bobwhites were euthanized with CO_2 inhalation followed by cervical dislocation and carcasses were frozen at −20 C until necropsy. All trapping and handling techniques were approved by the University of Georgia Animal Care and Use Committee (A2018 04-001 and A2020 11-010).

Bobwhites were carefully examined for ectoparasites, which were preserved in 70% ethanol (Doster et al. 1980). Ticks were identified to species using morphology as described (Thompson et al. 2022). Chewing lice (Mallophaga) and mites were identified using dichotomous keys and

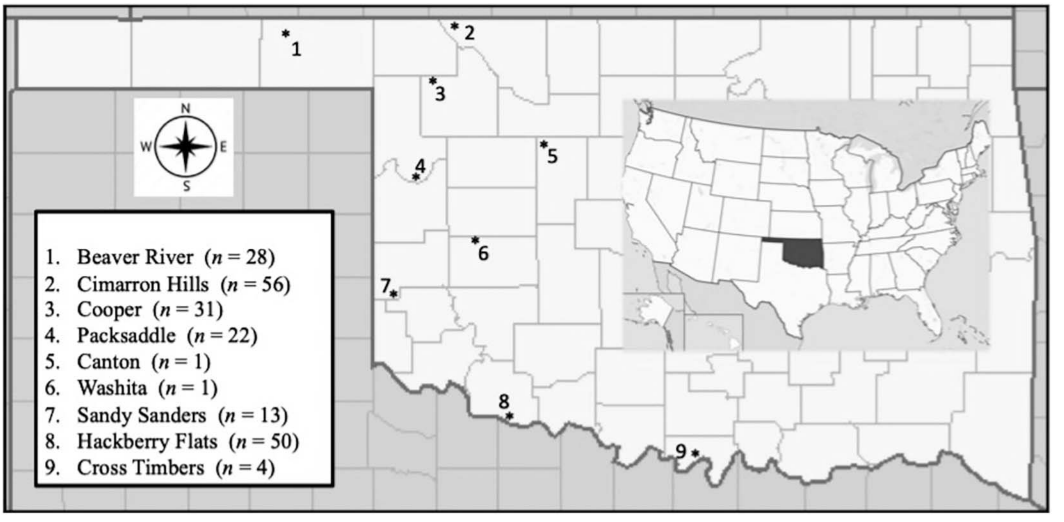

FIGURE 1. Wildlife management areas (WMAs) in Oklahoma, USA, sampled, and number of bobwhites (*Colinus virginianus*) sampled in 2018–20.

published literature (Brennan and Goff 1977; Price and Graham 1997; Price et al. 2003).

Bobwhites were initially classified as adult or juvenile age class by examining primary covert feathers for buffing; a more accurate age was determined by counting and measuring primary feathers undergoing growth and molt (Petrides and Nestler 1943). To evaluate nutritional condition, the amounts of breast muscle, subcutaneous fat, and visceral fat stores were subjectively scored from 0 to 6 (0=extreme emaciation and 6=obesity; Dunn 2003). Organs were examined for gross lesions; tissues containing any suspect lesions were placed in 10% neutral buffered formalin for histologic examination. For 20% of bobwhites from each WMA, pieces of heart, lung, kidney, liver, whole head (including brain and eyes), tongue, trachea, breast and leg muscles, spleen, pancreas, small intestine (duodenum and jejunum), cloacal bursa, cecal trident (including large intestine), gizzard, and proventriculus were collected for histologic examination. Head tissue data were included in an eyeworm study (Wyckoff et al. 2023). Formalin-fixed tissues were trimmed, embedded in paraffin, sectioned at 4 µm, and stained with H&E. Slides were blindly evaluated by a board-certified veterinary pathologist.

Additional samples were collected for targeted pathogen screening (Supplementary Material Tables S1 and S2). These samples were frozen to −20 C before DNA extraction. The gastrointestinal tract was removed and examined for parasites (reported in Wyckoff et al. 2023). For birds from which blood had not been previously collected from the jugular vein, clotted heart blood was collected and frozen for *Toxoplasma gondii* serologic testing.

Molecular and serology assays

Bobwhites were tested for selected pathogens by PCR or serologic assays described in Supplementary Material Tables S1 and S2. For PCR, genomic DNA was isolated using a commercial kit (DNEasy Blood and Tissue kit, Qiagen, Germantown, Maryland, USA) and PCR and sequencing methods were conducted as described (Chong et al. 2023). Precautions were taken to prevent and detect contamination, including performance of DNA extraction, PCR reaction setup, and product analysis in separate laboratory areas. Negative (water) controls were included in each batch of DNA extractions and for each set of PCR reactions. An appropriate positive control for each pathogen was included in each PCR batch. Phylogenetic analyses for haemosporidian and *Physaloptera* spp. nematode sequences were carried out using MEGA11 (Stecher et al. 2020). Consensus sequences obtained from this study were aligned with related sequences from GenBank (Sayers et al. 2023) and a phylogenetic tree was constructed using maximum likelihood algorithms using the Tamura-Nei–parameter model and pairwise deletion. Novel sequences were submitted to GenBank (accession nos. OP589137–OP589150).

Virus isolation for WNV

Isolation of flaviviruses from a sample of pooled heart, kidney, and brain tissues was conducted in confluent, 2-d-old Vero cell culture monolayers as described (Allison et al. 2004). Any samples that displayed cytopathic effects within 10 d were further evaluated using VectorTest WNV Antigen Assay (VecTOR Test Systems, Inc, Thousand Oaks, California, USA) to test for WNV antigen. If results indicated the presence of WNV antigen, a reverse-transcription PCR was conducted to confirm the presence of WNV RNA as described (Supplementary Material Table S1).

Gross parasite assessment

The breast muscles of all bobwhites were examined grossly for intramuscular parasites by checking the surface of the breast muscle, then carefully examining thin sections of the pectoralis and supracoracoideus muscles. The leg muscles were similarly examined. Any parasites found were stored in 70% ethanol. When multiple intramuscular parasites were observed in the same tissue, some parasites were excised along with surrounding tissue and stored in 10% buffered formalin for histologic examination. Once the intramuscular nematodes were morphologically identified as *Physaloptera* sp. (Boggs et al. 1990), genetic characterization was conducted to better characterize these parasites (Supplementary Material Table S1).

Toxicology testing

A subsample of 21 whole livers were submitted to the California Animal Health and Food Safety Laboratory at the University of California Davis (Davis, California, USA) for screening for heavy metals, including arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), lead (Pb), and zinc (Zn), and organic chemicals. Analysis for heavy metals was conducted as described (Ganoe et al. 2021), with detection limits of 1 ppm for Fe, As, Pb, and Hg; 0.4 ppm for Mo; and 0.3 ppm for Zn, Cu, and Cd. All results were reported on a tissue wet weight basis. The organic chemical screens were performed using a combination of gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry as described (Filigenzi et al. 2011; Ganoe et al. 2021). These screens are designed to detect hundreds of diverse organic compounds from different chemical categories, including pesticides, environmental contaminants, drugs, and natural products.

RESULTS

From 2018 to 2020, 206 bobwhites from nine sites, with a range of 1–56 bobwhites per WMA and 42–91 bobwhites per year, were sampled (Fig. 1). Similar numbers of adults (53%, n=110) and juveniles (47%, n=96) and females (50%, n=103) and males (50%, n=103) were sampled.

General health examinations

Overall, based on muscle mass and fat stores, adult bobwhites were in good nutritional condition with mean scores of 4.8±0.96 (standard deviation) for muscle mass and 3.4±1.47 for fat stores. Breast muscles were convex, indicating ample muscle mass and leaving the sternal keel bone unexposed (i.e., no protuberance). No bobwhite had concavity to the breast muscles, indicating that malnutrition and muscle atrophy were rare or absent in these populations during the sampling period. The fat store scores of adult bobwhites collected from the earlier trapping seasons (n=41, 3.37±1.3) were similar to the fat store scores of adult bobwhites sampled from the later trapping season (n=69, 3.41±1.57). Juvenile bobwhites were in good nutritional condition based on muscle mass and fat stores, with mean nutrition scores of 3.6±0.87 for muscle mass and 2.3±0.98 for fat stores. The weights of male and female adult bobwhites varied slightly year to year, although the overall mean body weights of the two sexes were nearly identical (181.1 and 180.8 g, respectively; Table 1).

No abnormalities or significant findings, such as those that may occur with avian pox, highly pathogenic avian influenza, gastroenteritis (e.g., clostridial), sinusitis, or conjunctivitis, were observed during gross necropsy of bobwhites.

TABLE 1. Mean weights of adult male and female bobwhites (*Colinus virginianus*) sampled in western Oklahoma, USA, 2018–20.

	Mean body weights ±SD (g)			
Adult bobwhite	2018 (37, 22)[a]	2019 (23, 13)[a]	2020 (10, 5)[a]	Overall (69, 39)[a]
Males	178.1±15.5	185.9±11.7	181.5±7.6	181.1±13.8
Females	179.5±17.8	183.5±11.1	180±6.2	180.8±14.6
Overall	178.6±16.2	185±11.4	180.7±6.9	181±14

[a] Sample sizes for males and females, respectively.

One bobwhite had a localized, mild fungal infection in the eyelid, and another had a discolored beak; neither of these findings was considered to be significant.

Bacterial and viral pathogen testing

Testing for WNV included both serology and virus isolation. No viruses were isolated from pooled tissues from the 206 bobwhites tested, and antibodies were not detected in 38 bobwhites tested. Antibodies to group I serotype 1 avian adenovirus (quail bronchitis virus) were detected in 4/46 (8.6%) bobwhites; all were adults (Table 2). None of the 206 bobwhites tested by PCR for reticuloendotheliosis virus had proviral DNA detected. No Mycoplasmataceae or *Pasteurella multocida* DNA was detected in 133 bobwhite tracheal swabs tested in 2019 and 2020.

Parasite testing

Endoparasites: A *Physaloptera* sp. was observed in the breast and leg muscles of 7–12% of bobwhites from 2018 to 2020 (Table 3 and Fig. 2A). Intensities (range 1–36 observed parasites, mean 5.2) decreased from a mean high of 7.8 in 2018 to 3.2 and 3.6 in 2019 and 2020, respectively (Table 3). Genetic characterization of 20 *Physaloptera* larvae revealed 14 unique sequences (550 bp); intraspecific variation was low (98.5–99.8% identical; genotypes A–N in Fig. 3). Four worms had identical sequences (genotype A); two additional worms were identical (genotype B); three additional worms were identical (genotype C) and the remaining worms all differed by at least one nucleotide (genotypes D–N). The sequences were most similar (97.7–98.9% identical) to a *Physaloptera* sp. previously detected in a bobwhite and grasshoppers (Orthoptera) from Mitchell County, Texas, US (Kalyanasundaram et al. 2018). None of the sequences of *Physaloptera* from definitive hosts in GenBank were close enough to consider them the same species, but the next closest match was a *Physaloptera* from a feral cat in India (no. MW517846; 97% identical).

Single *Sarcocystis* cysts were observed grossly during necropsy in 2/206 (1%) bobwhites, although PCR testing of 206 muscle samples yielded negative results for *Sarcocystis* (Table 3). We did not conduct PCR on the two sarcocysts observed grossly as they had been preserved in formalin for histologic examination. During gross and/or microscopic examination, *Tetrameres* spp. were observed in the proventriculus (Fig. 2B), with prevalences of 0%, 2%, and 10% in 2018, 2019, and 2020, respectively. *Tetrameres* spp. intensities ranged from 1 to 15 with a mean intensity of 5±5.2. Histologically, the *Tetrameres* spp. were not associated with any inflammation.

Three of 133 (2.3%) bobwhites had antibodies to *T. gondii* (Table 2). None of the 133 bobwhites tested for *Trichomonas* spp. and *Eimeria* spp. were positive. Eyeworms and gastrointestinal parasites were observed during necropsy; these results are reported elsewhere (Wyckoff et al. 2023).

TABLE 2. Results of molecular and serologic testing for selected pathogens in bobwhites (*Colinus virginianus*) from western Oklahoma, USA, in 2018–20.[a]

Pathogen	Pathogen type	Test method	No. infected bobwhites/No. sampled (%)			
			2018	2019	2020	Overall
Avian adenovirus	Virus	Serology (ELISA)	n/a	3/15 (20)	1/31 (3)	4/46 (8.6)[b]
Reticuloendotheliosis virus	Virus	PCR	0/73 (0)	0/91 (0)	0/42 (0)	0/206 (0)
West Nile virus	Virus	Isolation or serology (PRNT)[c]	0/73 (0)	0/91 (0)	0/42 (0)	0/206 (0)
Mycoplasmataceae species	Bacteria	PCR	n/a	0/91 (0)	0/42 (0)	0/133 (0)
Pasteurella multocida	Bacteria	PCR	n/a	0/91 (0)	0/42 (0)	0/133 (0)
Eimeria spp.	Protozoa	PCR	n/a	0/91 (0)	0/42 (0)	0/133 (0)
Haemoproteus spp.	Protozoa	PCR	1/62 (1.6)	2/90 (2.2)	0/42 (0)	3/194 (1.5)
Leucocytozoon spp.	Protozoa	PCR	0/62 (0)	0/90 (0)	3/42 (7.1)	3/194 (1.5)
Plasmodium spp.	Protozoa	PCR	1/62 (1.6)	5/90 (2.2)	1/42 (2.3)	7/194 (3.6)
Toxoplasma gondii	Protozoa	Serology (MAT)	n/a	1/91 (1)	2/42 (5)	3/133 (2.3)[b]
Trichomonas spp.	Protozoa	PCR	n/a	0/91 (0)	0/42 (0)	0/133 (0)

[a] PRNT = plaque reduction neutralization test; n/a = ? ?; MAT = modified agglutination test.
[b] All avian adenovirus positives were adults and all *T. gondii* positives were juveniles.
[c] Only 38 bobwhites were tested by serology.

TABLE 3. Intramuscular and intraproventricular parasites detected in bobwhites (*Colinus virginianus*) from western Oklahoma, USA, in 2018–20.

Parasite species	Location of infestation	No. infected bobwhites/No. sampled (%)				Range of intensity	Overall mean intensity	Total recovered
		2018	2019	2020	Overall			
Sarcocystis spp.	Breast muscle	0/73 (0)	2/91 (2)[a]	0/42 (0)	2/206 (1)	1	1	2
Physaloptera spp.	Breast and leg muscle	9/73 (12)	12/91 (13)	3/42 (7)	24/206 (12)	1–36	5.2±7.3[b]	114
Tetrameres spp.	Proventriculus	0/73 (0)	2/91 (2)	4/42 (10)	6/206 (3)	1–15	5±5.2	29

[a] Observed grossly.
[b] Annual range and mean intensity (±SD): 2018 (1–36, 7.8±11.2); 2019 (1–13, 3.1±3.2); and 2020 (1–15, 3.6±4.9).

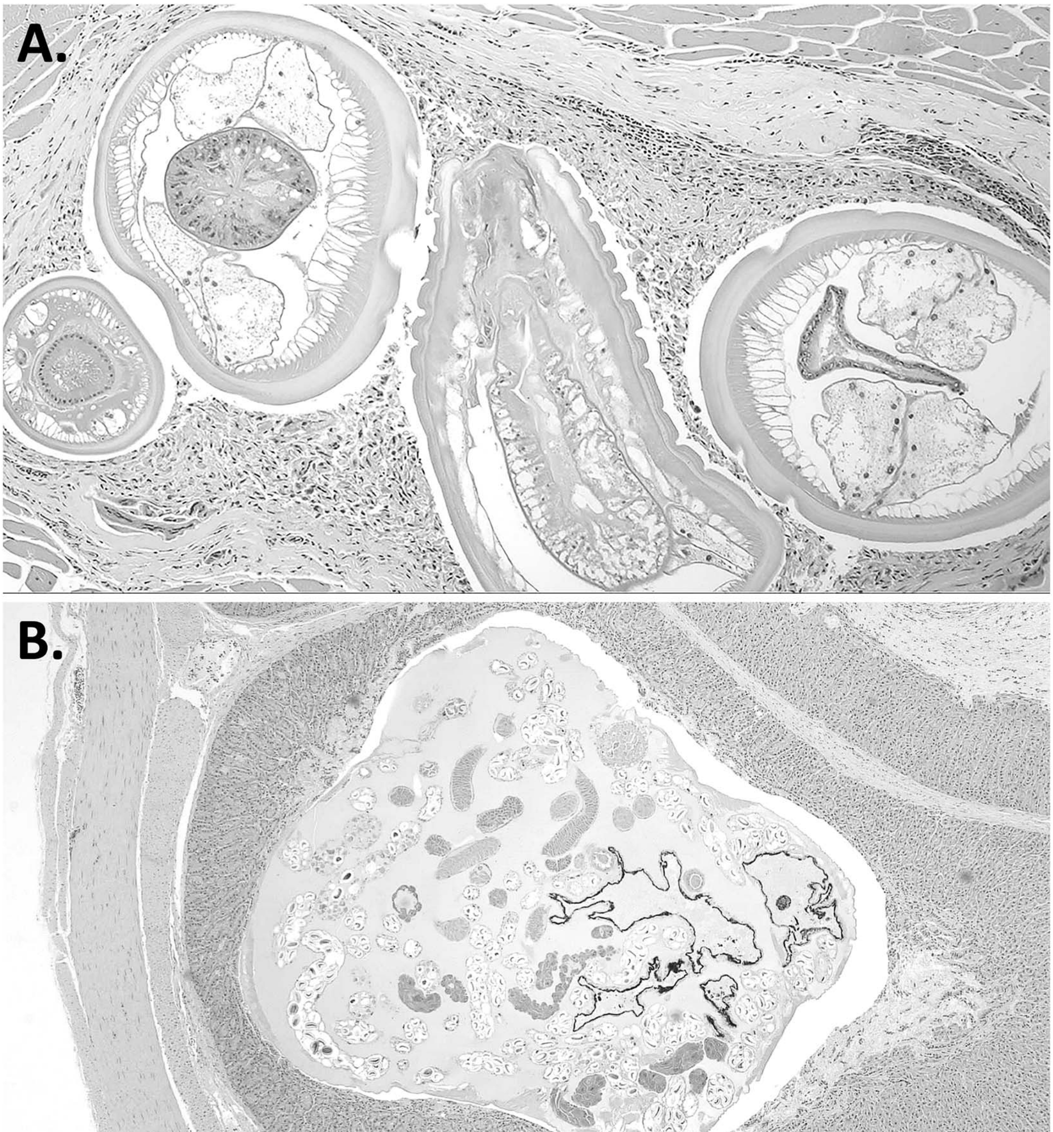

FIGURE 2. Photomicrographs of *Physaloptera* sp. and *Tetrameres* sp. (A) *Physaloptera* sp. embedded in the skeletal muscle of the breast, with mild surrounding inflammation and an outer fibrous capsule, indicating chronicity. (B) *Tetrameres* sp. expanding the glandular lumen of the proventriculus, with no associated inflammation.

Haemosporidian PCR testing: Based on the real-time screening assay, 21/194 spleen samples (10.8%) were PCR positive for blood parasites. Only 11 of these samples amplified with the nested PCR assays targeting either *Haemaproteus*-*Plasmodium* or *Leucocytozoon* spp. (Table 2). Sequence analysis indicated that seven (3.6%) were infected with *Plasmodium*, three (1.5%) with *Haemoproteus*, and three (1.5%) with *Leucocytozoon* spp. Two birds were coinfected with *Plasmodium* and *Leucocytozoon* spp. The *Haemoproteus* lineage detected was most similar to *Haemoproteus* sp. lineage AFR151 in the GenBank

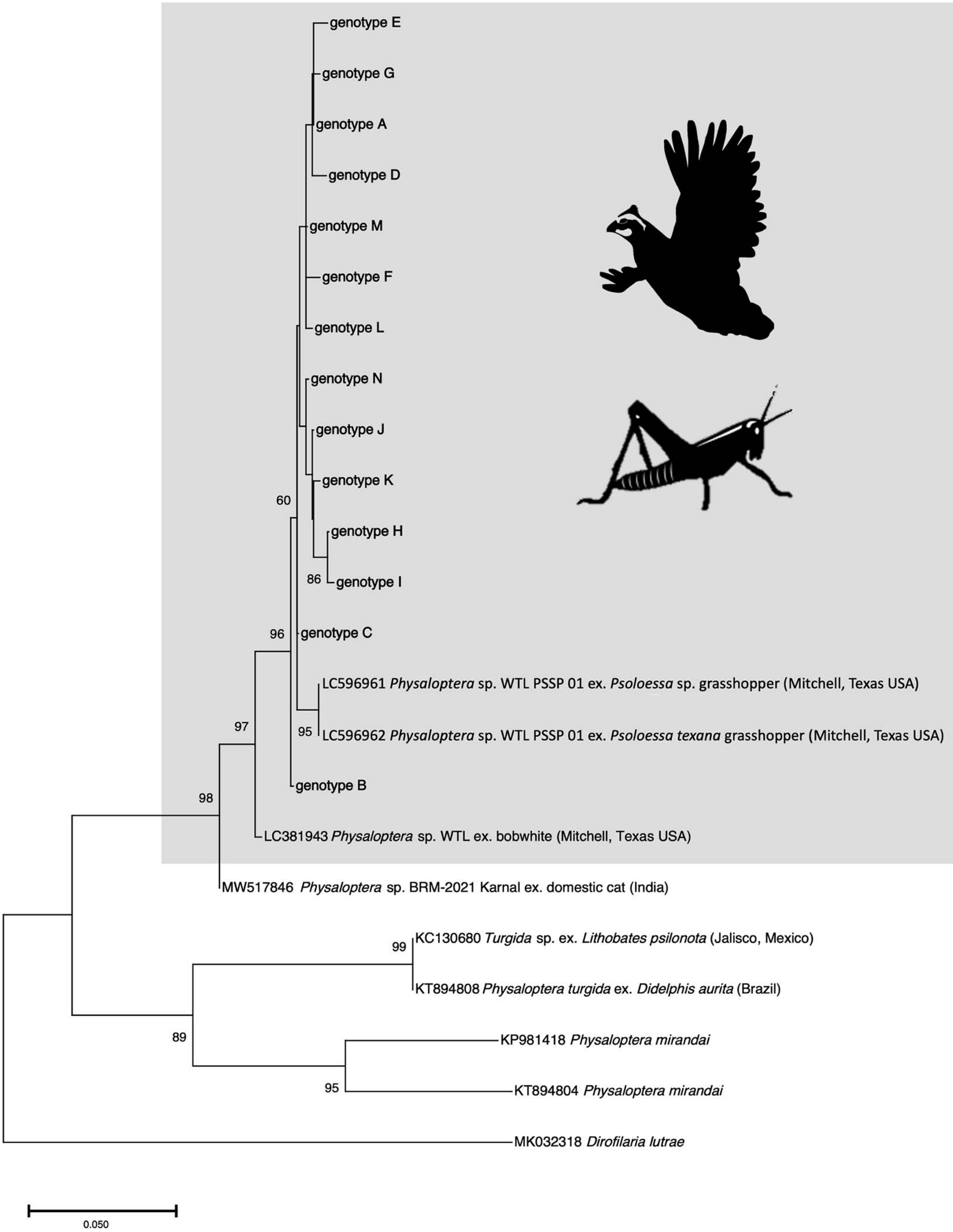

FIGURE 3. Phylogenetic analysis of *Physaloptera* (A–N) from bobwhite (*Colinus virginianus*) breast muscle tissue from Oklahoma, USA, 2018–20.

(483/491 bp) and Malavi (470/478 bp) databases. Phylogenetically, this lineage grouped separately from other related lineages as well as from *Haemoproteus* sequences from quail from the eastern US (Fig. 4). The *Plasmodium* sp. was 100% similar to *Plasmodium homopolare* haplotype 2 (lineage LAIRI01) and grouped with numerous other *P. homopolare* sequences,

FIGURE 4. Phylogenetic analysis of haemosporidians from bobwhites (*Colinus virginianus*) from Oklahoma, USA, 2018–20.

TABLE 4. Ectoparasites recovered from bobwhites (*Colinus virginianus*) in western Oklahoma, USA, in 2018–20.

Parasite species	Parasite type	No. infected bobwhites/No. sampled (%)				Range	Mean intensity ±SD	Total recovered
		2018	2019	2020	Overall			
Goniodes ortygis	Louse	8/73 (11)	49/91 (54)	24/42 (57)	81/206 (39)	1–11	2.6±2.3	213
Oxylipeurus clavatus	Louse	1/73 (1)	24/91 (26)	20/42 (48)	45/206 (22)	1–7	2.2±1.7	96
Menacanthus spp.	Louse	8/73 (11)	22/91 (24)	8/42 (19)	38/206 (18)	1–6	2.1±1.6	81
Trombiculidae species	Mite	1/73 (1)	7/91 (8)	4/42 (10)	12/206 (6)	1–3	1.3±0.6	17
Haemaphysalis leporispalustris	Tick	8/73 (11)	22/91 (24)	18/42 (30)	48/206 (23)	1–40	5.9±8.1	292
Amblyomma maculatum	Tick	2/73 (3)	15/91 (16)	5/42 (12)	22/206 (11)	1–25	4.7±6.1	94
Amblyomma americanum	Tick	0/73 (0)	2/91 (2)	0/42 (0)	2/206 (1)	1–3	2±1.4	4

including those from quail species in the western US (Fig. 4). The *Leucocytozoon* sequences (506 bp) were 96.8–97.1% similar to several sequences of *Leucocytozoon schoutedeni* from chickens (*Gallus gallus domesticus*) and black flies (Simulidae) in GenBank and were 100% similar to the *L. schoutedeni* lineage GALLUS23 in the MalAvi database. Phylogenetically, the bobwhite *Leucocytozoon* sequence grouped with numerous *L. schoutedeni* sequences (Fig. 4).

Ectoparasites: The prevalence of ectoparasites increased over time, with overall prevalences of 30% (n=73), 85% (n=91), and 98% (n=42) in 2018, 2019 and 2020, respectively. Three chewing lice species, *Goniodes ortygis*, *Menacanthus* sp., and *Oxylipeurus clavatus*, were collected, with *G. ortygis* (39%) being the most common species observed (Table 4). All occurred at low intensities. The only mites observed were chiggers (Trombiculidae) on 6% of quail.

We collected three tick species, *Amblyomma americanum*, *Amblyomma maculatum*, and *Haemaphysalis leporispalustris* (Table 4). The rabbit tick, *H. leporispalustris,* was the most prevalent tick, found on 23% of quail (total 266 larvae and 26 nymphs, range 1–40, mean 5.9). The Gulf Coast tick, *A. maculatum,* was the second most common species observed (15% prevalence, total 87 larvae and 7 nymphs, range 1–25, mean 4.7). Finally, the lone star tick, *A. americanum,* was rarely observed, with only four larvae detected on 2/206 (1.5%) bobwhites.

Toxicology testing

No toxins were detected in the livers of 21 bobwhites (Table 5). Livers of the 11 bobwhites screened for heavy metals had no evidence of As, Pb, Hg, or Cd, but low levels of Fe, Mn, Zn, Cu, and Mo were detected.

DISCUSSION

This study was conducted to generate baseline data on the diversity and prevalence of selected pathogens in bobwhites in western

TABLE 5. Toxin and heavy metal screening results for bobwhites (*Colinus virginianus*) from western Oklahoma, USA.

Toxicants	No. detected/total screened (%)	Average detection (ppm)	Range of detection (ppm)
Organic compounds	0/21 (0)	NA	NA
Lead	0/11 (0)	NA	NA
Manganese	11/11 (100)	3.5±1.0	2.4–4
Iron	11/11 (100)	217.3±103.5	140–510
Mercury	0/11 (0)	NA	NA
Arsenic	0/11 (0)	NA	NA
Molybdenum	10/11 (91)	3.9±2.4	1.3–8.7
Zinc	11/11 (100)	26.4±3.8	20–33
Copper	11/11 (100)	5.3±0.9	3.8–7
Cadmium	0/11 (0)	NA	NA

[a] Organic compounds (chemicals originating from pesticides, environmental; contaminants, drugs, and natural products).
[b] NA = not applicable.

Oklahoma. Overall, the sampled bobwhites were in good health with limited evidence of disease and few pathogens detected. However, several pathogens of concern (i.e., blood parasites, *T. gondii*, and adenovirus) were detected that warrant additional studies to understand any potential threats to bobwhite health.

The mean body weight of adult bobwhites remained relatively stable for the 3 yr of the study, suggesting that sufficient food and other resources were consistently available despite extreme weather that occurred in 2019 (near-record rainfall amounts) and 2020 (near-drought conditions) in western Oklahoma. In addition, the mean weights of both male and female bobwhites were similar to bobwhites sampled during a previous study also conducted in western Oklahoma (Andersson et al. 2021). Overall, both adult and juvenile bobwhites had good muscle mass and fat store scores. Juvenile bobwhites had significantly lower muscle mass and fat stores compared with adults, which was expected as juveniles undergo a period of rapid growth that uses a significant amount of energy. Additionally, adult bobwhites generally have a broader available prey base because of their larger body size and better foraging skills (Davidson et al. 1980; Dunham et al. 2016). In conjunction with weight data, evaluation of muscle mass and fat stores revealed that malnutrition and muscle atrophy were probably not a concern for quail populations in the area during the time frame of this study. This supports the notion that quail were able to forage adequately within the occupied habitat(s).

Low levels of the heavy metals Cu, Fe, Mn, Zn, and Mo were detected in bobwhite liver. All levels were considered normal and below safe levels for quail and other Galliformes (Donia 2015; Stafford et al. 2016). However, continued surveillance is warranted because these compounds can impact quail health, and we only tested a subset of bobwhites. Previously, Pb, Hg, and one organochlorine pesticide (*p,p′*-DDE) were detected in bobwhites from the Rolling Plains ecoregion of Texas and Oklahoma (Baxter et al. 2015); however, none of those were detected in our study.

The low prevalence of haemosporidians (*Haemoproteus*, *Leucocytozoon*, and *Plasmodium* spp.) is generally consistent with previous studies of wild bobwhites in Texas and Oklahoma (Kocan et al. 1979; Peterson 2007; Xiang et al. 2017). *Plasmodium homopolare* (haplotype 2, lineage LAIRI01) is a host generalist and has two haplotypes; H1 is widespread in the Americas and infects numerous bird species, primarily passerines and apodiforms (Pacheco et al. 2020), whereas H2 is more restricted to the western US and Galapagos Islands and has been reported from passerines, galliforms including Masked Bobwhite (*C. virginianus ridgwayi*) and raptors (Ishak et al. 2008; Pacheco et al. 2011, 2020; Keith et al. 2022; Chong et al. 2023). Lesions in captive Masked Bobwhite and Greater Sage-grouse (*Centrocercus urophasianus*) were potentially due to *H. homopolare* infection, which suggests this parasite can be pathogenic in certain species, although birds had coinfections with other haemosporidians (Pacheco et al. 2011; Chong et al. 2023). The *Haemoproteus* lineage was novel and distinct from *Haemoproteus* lineages from captive bobwhites and other quail in California, Utah, and Arizona (Cardona et al. 2002; Pacheco et al. 2011; Kelly et al. 2018). *Haemoproteus lophortyx* can cause serious illness and death in captive bobwhites (including Masked Bobwhite) quail and it has been detected in wild Gambel's (*Callipepla gambelii*) and Scaled Quail (*Callipepla squamata*) in Arizona, California, Colorado, Nevada, and New Mexico, US (Cardona et al. 2002; Peterson 2007). There are no sequences of *H. lophortyx* to compare with our sequences, and we did not have blood smears for morphology. Our *Leucocytozoon* lineage was similar to *L. schoutedeni,* a parasite of chickens in Africa and Asia and possibly the US (South Carolina; Sehgal et al. 2006). Because we did not have blood smears, the bobwhite *Leucocytozoon* sp. could not be compared morphologically with *L. schoutedeni.* Future studies should collect smears to allow detailed morphologic analyses. Blood parasites are of particular interest because environmental and climate change are altering the geographic ranges of these parasites and/or their vectors, which may affect disease risk.

There was no evidence of active or previous infection with WNV. A previous study in the Rolling Plains ecoregion of Texas found that a low percentage (4.8%) of wild-caught bobwhites had antibodies to WNV (Urban et al. 2013). Adult bobwhites experimentally infected with WNV developed subclinical infection with low viremia titers and readily seroconverted, suggesting bobwhites have low susceptibility to WNV-associated disease and are unlikely to play a role in mosquito-bird transmission cycles (Kunkel et al. 2021). However, susceptibility of bobwhite chicks has not been evaluated, and some age variation in manifestation of WNV infection has been observed in other bird species (Komar et al. 2003; Urban et al. 2013; Pérez-Ramírez et al. 2014).

A low prevalence of antibodies to *T. gondii* was detected. Bobwhites are highly susceptible to disease following experimental infection and may develop acute and chronic disease, often fatal. For example, 25% of bobwhites inoculated with a high dose of *T. gondii* died within a week (Dubey 2002; Dubey et al. 1993; Hill and Dubey 2002). Other quail species, such as California Quail (*Callipepla californica*) and Japanese Quail (*Coturnix japonica*), are also highly susceptible and may develop severe, sometimes fatal disease (Dubey et al. 1994; Casagrande et al. 2015). Despite the possibility of severe toxoplasmosis in bobwhites, there have been few surveys for *T. gondii* in wild populations, although toxoplasmosis has been diagnosed in wild quail from Georgia, US (Davidson et al. 1982). The lack of detection of oral *Trichomonas* spp. was similar to a study in Texas and Oklahoma, although bobwhites are experimentally susceptible to infection (but not disease) with this parasite (Levine et al. 1941; Bruno et al. 2015).

We detected antibodies to avian adenovirus, the causative agent of quail bronchitis, in nearly 9% of quail in Oklahoma, which is a potential health concern, especially for chicks. Quail bronchitis can develop in bobwhites of all age groups, and whereas adult bobwhites can survive infection, younger bobwhites often develop more severe disease, and chicks <3 wk of age may experience high mortality rates (e.g., 50–100%) (DuBose 1967; Jack and Reed 1990; Jack et al. 1994). Although there are several reports of avian adenovirus infection in captive bobwhites, data on free-ranging quail are limited. One study in Florida reported a prevalence of 23% for antibodies to avian adenovirus (King et al. 1981). Turkeys (*Meleagris galliparvo*) and chickens often are subclinically infected, which can facilitate the maintenance and spread of the virus in the environment (DuBose 1967; Jack and Reed 1990; Jack et al. 1994).

The ectoparasites detected are considered typical parasites on bobwhites (Peterson 2007). Although the overall prevalence increased during the study, ectoparasite intensities remained low. None of these ectoparasites are considered a health risk for bobwhites; however, *A. americanum* and *A. maculatum* are important vectors of pathogens of significance to people and animals (Lockwood et al. 2018; Paddock and Yabsley 2007). *Haemaphysalis leporispalustris* is not a recognized vector of human or veterinary pathogens, but this species must be distinguished from the invasive Asian longhorned tick (*Haemaphysalis longicornis*), as immature stages can look similar (Egizi et al. 2020; Thompson et al. 2020, 2022). The Asian longhorned tick, recently introduced to the eastern US, has been reported as far west in the US as northwestern Arkansas and Missouri; thus, monitoring its spread has important public and veterinary health implications.

The prevalence of *Tetrameres* spp. was similar to studies in Texas (1–26%) (Olsen et al. 2016; Villarreal et al. 2016; Herzog et al. 2021; Shea et al. 2021). Histologically, the infested proventricular glands were dilated with no associated cellular reaction or tissue damage. However, associated lesions (e.g., ulceration, severe inflammation) have been reported in the proventriculus when the parasites occur in high intensities (well above our observed maximum intensity of 15; Kellogg and Doster 1972).

Physaloptera sp. larvae have been reported in the breast muscle of bobwhites since the 1930s, and potential breast muscle damage has caused concern. We detected *Physaloptera* sp. larvae in 11% of quail, similar to previous studies in Oklahoma and Texas (4.8–23%; Boggs et al. 1990; Kalyanasundaram et al. 2018; Bruno et al. 2019; Herzog et al. 2021). We noted annual variation in mean intensity, which has also been noted by Kalyanasundaram et al. (2018) and may be related to intermediate host densities. We observed minimal microscopic inflammation in bobwhites, whereas a previous study in Oklahoma noted granulomatous inflammation and necrosis, although time since infection may impact presence of lesions (Boggs et al. 1990). The bobwhite serves as a paratenic host for *Physaloptera* sp., and to date no definitive host has been identified. Our *Physaloptera* sequences from Oklahoma bobwhites were similar to those from Texas (Kalyanasundaram et al. 2018), and although we found a high diversity of unique haplotypes, intraspecific variation was low. However, these new sequences failed to identify potential definitive host(s). The closest match available in GenBank was from a feral cat in India, but the bobwhite *Physaloptera* sp. sequences did not match any North American sequences, including sequences from *Physaloptera* spp. from raccoons, opossums, coyotes, dogs, and cats (Yabsley, pers. comm.). Molecular characterization of *Physaloptera* spp. from additional mammalian, raptor, and reptile hosts is needed to identify the definitive host. Additionally, studies on factors related to *Physaloptera* transmission are needed as higher intensities can probably cause more extensive muscle damage that could cause morbidity and increased predation risk.

Research on the health status of populations of bobwhites can provide critical data for selecting the best management strategies; knowledge of the diversity and prevalence of pathogens and parasites is important for understanding disease risk. Overall, free-ranging bobwhites in western Oklahoma were in good general health based on weights and fat stores and had a low diversity and prevalence of pathogens and parasites; however, some potential pathogens (e.g., adenovirus) and parasites (e.g., haemosporidian parasites, *T. gondii*) detected may be associated with health impacts in bobwhites, especially in chicks (Villarreal et al. 2016; Bruno et al. 2019; Herzog et al. 2021).

Disease in chicks is particularly hard to document because dead chicks are rarely found and submitted for diagnostic evaluation. Further investigations on potential population health risks are needed to assist in future disease surveillance and management strategies in bobwhites.

ACKNOWLEDGMENTS

This study would not have been possible without the hard work of the many Oklahoma Department of Wildlife Conservation (ODWC) biologists and technicians from the sampled Wildlife Management Areas. Support for this study came from the Oklahoma Department of Wildlife Conservation through federal aid, the Pittman-Robertson Wildlife Restoration Act under project W-204-R-1. Additional support was provided by the wildlife management agencies of the Southeastern Cooperative Wildlife Disease Study member states in Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Mississippi, Missouri, Nebraska, North Carolina, Oklahoma, South Carolina, Tennessee, Virginia, and West Virginia through the Federal Aid to Wildlife Restoration Act (50 Stat. 917), the United States Fish and Wildlife Service, and by a U.S. Department of the Interior Cooperative Agreement. We appreciate the efforts of skilled histotechnicians at the University of Georgia, Department of Pathology Histology Laboratory. We thank technicians at the Poultry Research and Diagnostic Center (University of Georgia) for laboratory support and Robert Poppenga and technicians at the California Animal Health and Food Safety Laboratory and toxicologic analyses.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-23-00102.

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Submitted for publication 26 June 2023.
Accepted 25 October 2023.