BASELINE HEALTH SURVEY OF NOTHERN BOBWHITE QUAIL (*COLINUS VIRGINIANUS*) ACROSS WESTERN OKLAHOMA

by

SETH TIMOTHY WYCKOFF

(Under the Direction of Michael J. Yabsley)

ABSTRACT

The Northern Bobwhite Quail (*Colinus virginianus*) is a valuable upland game bird in Oklahoma that has been suffering from severe and ongoing population decline. In this study, we evaluated the health of bobwhite quail across western Oklahoma through necropsy and screening for selected pathogens and parasites. A total of 206 bobwhite were sampled, processed, and were found to be in overall good condition with ample muscle mass and visible fat stores. Many pathogens screened for were not found; however, several pathogens or protozoan parasites of potential importance were detected including *Toxoplasma gondii* and Avian Adenovirus. Although numerous species of external, intramuscular, intraorbital and gastrointestinal parasites were observed, the prevalence and intensities were low. However, two parasites (*Aulonocephalus pennula* and Raillietina) were associated with reduced fat stores. Establishing baseline health data can help guide management strategies to preserve existing populations of the Northern Bobwhite Quail in Oklahoma.

INDEX WORDS: Northern Bobwhite Quail, health survey, population decline, parasites, pathogens, *Colinus virginianus, Oxyspirura petrowi*

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B.S., University of Georgia, 2017

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2021

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December 2021

ACKNOWLEDGEMENTS

First and foremost, I'd like to say thank you to my committee members, Drs. Michael Yabsley, Mark Ruder, Nicole Nemeth, and James Martin. Each committee member went above and beyond to address any questions or concerned I had. The guidance, support, and knowledge my committee provided was instrumental to success and completion of this project. I will forever be grateful for the opportunity to continue my education that was put forth by Michael and Mark.

I would like to acknowledge the Oklahoma Department of Wildlife Conversation (ODWC) for their contributions with funding and providing the bobwhite for study. A special thank you to the wildlife biologists, WMA managers, and my ODWC point of contact Tell Judkins for carrying out the majority of the fieldwork that this study required.

I would like to say a special thank you to other members of the Yabsley Lab, past and present for the help ranging from answering endless questions about PCR to simply reminding me on a monthly basis about the lab meeting that I most certainly forgot about. A particular thank you to Kayla Garrett for answering questions that I would ask daily, and Alec Thompson who went out of his way to teach me various molecular and serological techniques.

A special thanks to Kayla Adcock and Melanie Kunkel for their invaluable contributions. Without them, the comprehensive screening for West Nile virus and Reticuloendothelisosis virus would not have been possible.

And finally, I would like to acknowledge my friends and family for the continual love and support. A most heartfelt thank you to my fiancée, Liandrie Swanepoel, who pushed me to

pursue a graduate degree back when we were both just undergrads, and whose support has never wavered.

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CHAPTER 1

INTRODUCTION

The Northern Bobwhite Quail (*Colinus virginianus*), hereafter bobwhite, is one of the most influential game species in Oklahoma. This small galliform attracts hunters across the state of Oklahoma as well as numerous other states. The bobwhite has a rich history as a greatly sought-after upland game bird that generations of Oklahoman hunters have enjoyed pursuing and harvesting. Despite the popularity of the bobwhite, and the resources put towards their conservation, bobwhite populations throughout their historic range have been declining. Since the mid-1960's, the bobwhite population has decreased by an estimated 85% according to the North American Bird Breeding Survey (Sauer et al., 2013). Evidence of bobwhite population decreases have also been noted from annual roadside count surveys and decreasing number of hunter harvest bobwhite each year (Janus, 2018; Judkins, 2020b, 2020a). With bobwhite populations decreasing at nearly 4% a year across their entire range, research into their health and possible factors associated with population declines are needed (Hernández et al., 2013; ODWC, 2017; Sauer et al., 2013). Identifying and understanding significant mortality factors of bobwhite populations is important to not only conserve the species, but also preserve an integral part of outdoorsman culture.

Bobwhite are subjected to numerous natural pressures such as predation, disease, extreme weather patterns, food availability, and large-scale disturbances such as wildfires and flooding. There are also pressures affecting bobwhite that are anthropogenic such as climate change, exposure to toxicants, and habitat fragmentation through farming, ranching, road building, and urbanization (L. A. Brennan & Kuvlesky, 2005; Hernández et al., 2013). Even with all the known mortality factors, not a single one has been identified as the primary cause for the range wide population decline. Additionally, other unknown factors may be important. Studies have highlighted the importance of suitable habitat for bobwhite survival, and the negative impacts habitat destruction and fragmentation have had on local bobwhite populations (Forrester et al., 2000; Miller et al., 2019; Parent et al., 2016). In areas with suitable habitat that are still experiencing a decline in bobwhite numbers, the culprit for the decline is suspected to be heavy burdens and high prevalence of specific parasites such *as Oxyspirura petrowi* (Eyeworms) and *Aulonocephalus pennula* (Caecal worms) (Brym et al., 2018; Commons et al., 2019). While habitat suitability, parasitism, and disease are instrumental to bobwhite health, establishing and understanding health data of individual bobwhite can allow for more meaningful assessments of potential pathogen induced health risks to quail populations. The primary goal of this project is to create a baseline health survey of bobwhite quail across western Oklahoma. Objectives include establishing baseline health data, identifying external and internal parasites, screening for selected pathogens including West Nile virus, as well as determining the prevalence of *O. petrowi* and potential physiological effects their infections may have on bobwhite.

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CHAPTER 2

LITERATURE REVIEW

THE DECLINE OF NORTHERN BOBWHITE POPULATIONS

The nationwide population of Northern Bobwhite Quail (*Colinus virginianus*), hereafter bobwhite(s), have been undergoing a significant decline from the 1960's to the present day of over 75% (Leonard A. Brennan, 2006; Leonard A. Brennan & Kuvlesky, 2005; Hernández et al., 2013; Xiang et al., 2017). The bobwhite population has undergone an annual rate of decline of 4% from 1966 to 2017 (Dimmick, R.W., M.J. Gudlin, 2002; Sauer et al., 2013, 2017) across its entire historic range. Once abundant throughout their range, the bobwhite has become scarce in most of its endemic range (Arnold, 2019). This scarcity is clearly seen in areas of Oklahoma where bobwhite were once extremely accessible, but in recent decades, the number of harvest quail in Oklahoma has dropped from over 2.7 million birds in 1986 to 476,000 birds in 2008 (ODWC, 2017). The bobwhite scarcity has led to a decreasing number of registered hunters, from 111,000 in 1986 to 31,000 in 2008 (ODWC, 2017). Currently, bobwhite populations, bobwhite annually harvested in Oklahoma, and registered bobwhite permit holders in Oklahoma are still declining (ODWC, 2017). Although bobwhite numbers in Oklahoma are decreasing, the population decline is slower compared to other states in their range (Leonard A. Brennan, 2006), thus Oklahoma is currently a stronghold for bobwhite (Dabney & Dimmick, 1974).

Despite being one of the most intensely studied upland game birds and receiving considerable research attention over the last 50 years, a definitive reason for the continual bobwhite population decline has yet to be found (Duquette, Cameron; Davis,Craig;

Fulendorf,Samuel; Elmore, 2019; Fidel Hernández , Fred S . Guthery, 2019; Guthery, 1997). While various potential factors have been considered, no single factor has been definitively concluded to be the singular cause of the population decline (Arnold, 2019).

Appropriate habitat is crucial to bobwhite survival with the need for cover to avoid predation and construct nests (COX et al., 2004; Guthery, 1997; ODWC, 2017; Rollins & Carroll, 2001). Habitat loss is thought to be one of the leading causes for loss of biodiversity in North America (Pimm & Raven, 2000). While habitat requirements for bobwhite are not strict, they do prefer tall grasses, woody shrubs, and orbs for cover and nest construction (Leonard A. Brennan & Kuvlesky, 2005; Guthery, 1997; Hernández & Peterson, 2007). Historically, if there is open canopy, a sparse woody mid-story, and a diverse groundcover layer, bobwhites may be abundant (Miley & Lichtler, 2009). As human populations, road density, and pastural farmland have increased overtime, a decrease in bobwhite population numbers have been noted (Miller et al., 2019). It has been observed that the likelihood of bobwhite mortality decreases as the bobwhite's proximity to roads decreases (Tanner et al., 2016). The ongoing decrease in bobwhite population numbers concurrent with increased anthropomorphic landscape changes may be in part attributed to the negative impacts of habitat fragmentation (Dailey, Tom; Hutton, 2007; Miller et al., 2019).

The introduction of monoculture fields and decreasing number of woody shrublands, have had negative effects on bobwhite populations (Dailey, Tom; Hutton, 2007; Miller et al., 2019; Pierce et al., 2014). These monoculture croplands not only reduce year-round cover due to seasonal harvesting, but also a lack of food diversity for bobwhite (Miller et al., 2019; Pierce et al., 2014). Appropriate habitat cover is absolutely essential to bobwhite survival (Leonard A. Brennan & Kuvlesky, 2005; N. D. Forrester et al., 2000). Nests, chicks, and adults without proper cover are more susceptible to predation from a multitude of terrestrial and aerial predators (COX et al., 2004; Dailey, Tom; Hutton, 2007; ODWC, 2017; Rollins & Carroll, 2001). With complete removal of available flora associated with annual monoculture crop harvest, the habitat is left barren and void of much needed food and cover, leaving the bobwhite to struggle even harder with over-wintering starvation and freezing temperatures (Dabbert et al., 1997; Errington, 1939; N. D. Forrester et al., 2000; Miller et al., 2019; Parent et al., 2016; Rogers, 1987).

While commercial croplands may be the leading contributor to habitat fragmentation of bobwhite, the clearing of tall grasses and shrublands for cattle grazing is nearly as impactful (Leonard A. Brennan & Kuvlesky, 2005; Parent et al., 2016). Aside from simply clearing the land to better accommodate free-ranging livestock, the grazing from livestock quickly exceeded the carrying capacity of naturally occurring flora, reducing available food and resources for bobwhite (Leonard A. Brennan & Kuvlesky, 2005; Fidel Hernández , Fred S . Guthery, 2019). With tall grasses, shrubs, and orbs removed from the landscape, the occurrence of natural disturbance of wildfires is diminished (Leonard A. Brennan & Kuvlesky, 2005; Engstrom et al., 1984). Bobwhite have been noted to undergo a decrease of population growth in the absence of fire disturbances (Engstrom et al., 1984). Naturally occurring wildfires and prescribed burns are both credited with clearing ground litter, increasing successional flora, decreasing parasite burdens, and increasing insect populations; all of which benefit bobwhite (L. A. Brennan et al., 1998; Cram et al., 2019; Martin et al., 2018).

Anthropological landscape changes are not limited to building homes, clearing natural habitats for croplands and pastures, or introducing artificial breaks in cover in the form of roads, but also the introduction of oil and gas infrastructure. While oil and gas infrastructures contribute less to habitat fragmentation than other anthropogenic landscape changes previously listed, they do have indirect impacts such as increased noise, traffic, light pollution, altered fearscape (i.e change of provided cover which affects prey visibility and concealment) (Olsoy et al., 2015; Tanner et al., 2016). Despite the unnatural disturbances that oil and gas infrastructure pose, several studies have found that the effects of the these infrastructures have had a minimal effect on bobwhite populations (Duquette, Cameron; Davis,Craig; Fulendorf,Samuel; Elmore, 2019; Tanner et al., 2016).

Croplands after often treated with insecticides that are harmful to nonmammalian species, including bobwhites (Ertl et al., 2018; Gobeli et al., 2017). 80% of these insecticides fall into the broad category of neonicotinoids (Jeschke et al., 2011), which are the globally the most prevalent insecticides (Gobeli et al., 2017). Neonicotinoids are a potent neurotoxin used in agricultural for their extreme toxicity to pest insects (Goulson, 2014). Avian mortalities have been documented due to neonicotinoid intoxication (Abu Zeid et al., 2019; Berny et al., 1999). Pesticides not only reduce food availability to bobwhite, but when seeds coated with neonicotinoids such as imidacloprid, direct effects of decreased locomotory has been observed in birds (Abu Zeid et al., 2019). In an experimental study, imidacloprid was found to have potential developmental effects in bobwhite embryos (Gobeli et al., 2017). Another common group of pesticides is organophosphates, which are used for their short persistence in the environment and reduced accumulation in food chains (Galindo et al., 1985). While organophosphate pesticides pose less of threat of persisting in the environment, they are acutely toxic to wildlife (Galindo et al., 1985). The organophosphate pesticide (O,O-dimethyl o-p-nitrophenyl phosphorothioate) was found to reduce activity of bobwhite by 42% at sub-lethal doses, making the bobwhite more susceptible to predation (Galindo et al., 1985).

Predation is a common occurrence for bobwhite as they are near the very bottom of the food chain, with predation making up over half of the annual bobwhite mortalities (Dailey, Tom; Hutton, 2007; Rollins & Carroll, 2001). While parasites and disease primarily afflict adult birds (A. Bruno et al., 2019; Villarreal et al., 2016), most mortalities of juvenile birds are associated with predation (Rollins & Carroll, 2001). Nests, chicks, and adults without proper cover are more susceptible to predation (COX et al., 2004; Dailey, Tom; Hutton, 2007; ODWC, 2017; Rollins & Carroll, 2001). Predators of bobwhite eggs, chicks, and adults include Striped Skunks (*Mephitis mephitis*), Opossums (*Didelphis viriginianus*), Grey foxes (*Urocyon cinereoargenteus*), Red Foxes (*Vulpes vulpes*), Raccoons (*Procyon lotor*), Cotton Rats (*Sigmodon sp.*), various mustilids, Bobcats (*Lynx rufus*), Ground squirrels (*Otospermophilus beecheyi*), Fox squirrels (*Sciurus niger*), Gray squirrels (*Sciurus carolinensis*), Cooper's hawks (*Accipiter cooperii)*, Sharp-shinned hawks (*Accipiter striatus*), Red-tailed hawks (*Buteo jamaicensis*), Marsh hawks (*Circus cyaneus*), Great-horned owls (*Bubo virginianus*), Barred owls (*Strix varia*), Blue-jays (*Cyanocitta cristata*), American crows (*Corvus brachyrhynchos)*, wild turkey (*Meleagris gallopavo*), several species of snakes, domestic cats and dogs (*Felis catus* and *Canis lupus familiaris*), wild hogs (*Sus scrufa*), and even Ornate-Box turtles (*Terrapene ornata ornate*) on occasion (COX et al., 2004; Dailey, Tom; Hutton, 2007; ODWC ,2017; Rollins & Carroll 2001). With less than a 50% hatch rate in Oklahoma, 82% of nest depredation is carried out by raccoons (Rollins & Carroll, 2001). Roughly 70% of adult bobwhite mortality is attributed to mammalian and raptorial predators (Rollins & Carroll, 2001).

Although many studies have investigated parasites in bobwhites and there are some studies that suggest parasitism is associated with bobwhite population declines, this topic remains controversial (Brym et al., 2018b, 2018a; Nicholas R. Dunham, Peper, et al., 2017;

Hudson et al., 1998; Olsen et al., 2016). Historically, parasites were not considered to be of real significance to the health of bobwhite (Davidson et al., 1982), but recent studies in Texas suggest that some parasites may have population level impacts (Olsen et al., 2016). Parasites are a major biotic agent that can kill individual bobwhites (direct effect) or cause morbidity, making individuals more susceptible to predation (indirect effect) (Xiang et al., 2017).

Bobwhites are hosts for blood-borne protozoan parasites including several species of *Haemoproteus*, *Luecocytozoon*, and *Plasmodium* (Hellgren et al., 2004; F. Kellogg & Calpin, 1971). While these protozoan parasites are not suspected to cause major health impacts to bobwhite, they can have negative health impacts on individual bobwhites (Cardona et al., 2002). Parasites such as ticks, mites, and lice are generally considered to be benign and generally not detrimental to bobwhite health (F. Kellogg & Calpin, 1971). However, there are two parasites, *Oxyspirura petrowi* and *Aulonocephalus pennula,* that are currently suspected contribute to regional bobwhite population declines in Texas (Brym et al., 2018a; Olsen et al., 2016). With reports of increasing of parasitic burdens of *O. petrowi* and *A. pennula* and decreasing bobwhite populations, these correlations are investigated with hopes to find much needed answers (Blanchard et al., 2019; Nicholas R. Dunham, Bruno, et al., 2016; Nicholas R. Dunham, Reed, et al., 2016; Villarreal et al., 2012). Because limited work has been done regarding parasite burdens in Oklahoma, research is needed to better understand the diversity and burdens of parasites infecting bobwhites in this region.

PARASITES AND PATHOGENS ASSOCIATED WITH BOBWHITE QUAIL

Protozoan parasites

Protozoa are unicellular eukaryotic organisms that can be free-living in the environment or parasites of hosts (Yaeger, 1996). With > 50,000 species of protozoa inhabiting nearly every possible ecosystem, most animals are infected with one or more species of protozoa. Protozoa are divided into four major categories; Sarcodina (i.e. amoeba), Flagellata (i.e. *Trichomonas*), Sporozoa (i.e. *Sarcocystis*), and Infusoria (i.e. Ciliates) (Omarova et al., 2018; Peek, 2010). Infections of protozoa can range from benign and asymptomatic to life threatening depending on the species of protozoa and the immune system response of the host (Smith, 1996; Yaeger, 1996). Protozoa can be found living in a variety of tissues and systems, such as blood, muscle, gastrointestinal tract, and the brain (Dubey, 2002; Q.-Q. Li et al., 2002; Smith, 1996).

Haemoproteus, a genus of vector-transmitted protozoan parasites in the family Haemoproteidae, infects the red blood cells in numerous bird species, including bobwhite (Atkinson, 2008b; Soares et al., 2016). First described in 1890 by Kruse, 196 species have been described, although numerous lineages of *Haemoproteus* spp. remain undescribed (Bennett et al., 1993; Desser & Bennett, 1993; Garvin et al., 2003). Avian species are infected with *Haemoproteus* spp. by biting midges in the *Ceratopogonidae* family and louse flies in the *Hippoboscidae* family (Santiago-Alarcon et al., 2012). In addition, numerous mosquito genera (*Culex, Anopheles, Aede*s, and *Coquillettidia*) are shown to be viable vectors experimentally, although their role in nature is unknown ((Gutiérrez-López et al., 2016; Santiago-Alarcon et al., 2012).

Haemoproteus spp. are present in avian species worldwide, with some species causing pathologic effects such as myositis, decrease in growth, anemia, anorexia, ataxia, predation avoidance, reduced reproductive success, and death (Himmel et al., 2019; Jiménez-Peñuela et al., 2019; Nourani et al., 2018; Pacheco et al., 2011). Infection with *Haemoproteus* spp. can be influenced by factors such as age, sex, habitat, altitude, and the host immune system (Nourani et al., 2018). In most natural hosts, health impacts due to *Haemoproteus* infections are often consider non-life threatening but capable of compounding the effects of other stressors on avian health (Cardona et al., 2002; Jiménez-Peñuela et al., 2019; Santiago-Alarcon et al., 2012). Detection of *Haemoproteus* can be carried out by examination of Giemsa stained thin blood smears for infected erythrocytes or by molecular screening (Hellgren et al., 2004; Himmel et al., 2019; Xiang et al., 2017).

Plasmodium is another genus of vector-transmitted protozoan parasites, belonging to the family of Plasmodiidae which also infect red blood cells (Bennett et al., 1993; Soares et al., 2016; Valkiunas & Iezhova, 2017, 2018). *Plasmodium* spp. are a cosmopolitan group of parasites, infecting vast variety of avian species across the world (Pacheco et al., 2011; Valkiunas & Iezhova, 2017, 2018). Avian species are infected with *Plasmodium* spp. by culicidae mosquitos (*Culex, Coquillettidia*, *Aedes, Mansonia, Culisetta*, *Anopheles, Psorophora*) (Gutiérrez-López et al., 2016; Nay et al., 1982; Santiago-Alarcon et al., 2012; Valkiunas & Iezhova, 2018). *Plasmodium* sp. infections can range from asymptomatic to severe in disease presentation, and even be lethal to some avian species (Himmel et al., 2019; Peterson, 2007; Valkiunas & Iezhova, 2017). Bobwhite are susceptible to some *Plasmodium* sp. and can even act as a reservoir host for some. (Nay et al., 1982).

In rare instances, *Plasmodium* sp. can have devasting effects on naïve avian species through the introduction of invasive arthropod vectors, resulting in mass mortalities (Atkinson, 2008a). Hawaii's native avifauna is currently undergoing *Plasmodium reticlum* infections, with potential extinction to some avian species, due to the introduction of *Culex quinquefasciatus* (Jarvi et al., 2008; Liao et al., 2017). Detection of *Plasmodium* can be by examination of Giemsa stained thin blood smears for infected erythrocytes or by molecular screening (Hellgren et al., 2004; Himmel et al., 2019; Xiang et al., 2017).

Leucocytozoon is another genus of vector-transmitted protozoan parasite, belonging to the family of Leucocytozoidae, infecting the red blood cells (Bennett et al., 1993; Soares et al., 2016; Valkiunas & Iezhova, 2017). *Leucocytozoon* was initially described by Visily Danilewsky in 1888, and furthered described by N. Sakharoff in 1893. Since then, over a 100 species have been described from over 100 avian species (Valkiunas, 2004). *Leucocytozoon* spp. are found nearly world-wide in North America, Europe, Africa, and Asia (D. J. Forrester & Greiner, 2008). The *Leucocytozoon* genus was originally named as such due to the belief that they only infected leukocytes, later it was described that they infect and develop in erythrocytes as well (Desser, 1967).

The arthropod vector for *Leucocytozoon* spp. are black flies in the family *Simuliidae* (Lotta et al., 2016; Valkiunas, 2004). Severity of disease associated with *Leucocytozoon* sp. infections range from benign to severe disease (Lotta et al., 2016; Valkiunas, 2004). Detrimental effects of heavy infections of *Leucocytozoon* sp. include impacts to fitness, reproductive success, and even death (Bennett et al., 1993; D. J. Forrester & Greiner, 2008; Lotta et al., 2016). Avian groups that most often develop disease include waterfowl, pigeons, galliforms, raptors, and even ostriches (Desser & Bennett, 1993; D. J. Forrester & Greiner, 2008). Detection of *Leucocytozoon* can be carried out by examination of Giemsa stained thin blood smears for infected erythrocytes or by molecular screening (Hellgren et al., 2004; Himmel et al., 2019; Xiang et al., 2017).

Intestinal coccidian in the genera *Eimeria* can cause disease in high diversity of avian species, especially young birds (Duszynski & Gutiérrez, 1981; Greiner, 2008; Xiang et al., 2017). Numerous species of *Eimeria* have been found in the intestinal tract of bobwhite (Duszynski & Gutiérrez, 1981; Xiang et al., 2017; Yabsley, 2008) Coccidiosis has a well-established history as a disease, being reported as far back as the early 1800's (Yabsley, 2008). Coccidiosis is a parasitic disease that mainly affects the intestinal tract (Peek, 2010). Coccidiosis can cause large mortality events in large flocks, groups, or coveys in high densities (Peek, 2010). Coccidiosis causes diarrhea in most species, commonly observed as white dysentery in game birds (Boughton, 1937; Sneed & Jones, 1950). Symptoms of coccidiosis in avian species vary by host species, age, and coinfections. Symptoms include, but are not limited to, emaciation, diarrhea, listlessness, lack of appetite, dehydration, and mortality (Boughton, 1937; Musaev et al., 1998; Peek, 2010). Young bobwhite tend to be more susceptible to coccidiosis than adults (Davidson et al., 1982; Ruff, 1985; Sneed & Jones, 1950; Xiang et al., 2017; Yabsley, 2008). Detection of coccidians is via molecular methods or by fecal floatation of feces (Boughton, 1937; Q.-Q. Li et al., 2002; Sneed & Jones, 1950).

Trichomonosis is a disease of the respiratory and upper digestive tract caused by the flagellated protozoan parasites in the genus *Trichomonas* (D. J. Forrester & Foster, 2008). Trichomonosis is often credited as the oldest recorded wildlife disease, as it was described in the book 'The Book of Faulconrie or Hawking' by G. Tubervile in 1575 (D. J. Forrester & Foster, 2008). It wasn't until 1878 that S. Rivolta first described the etiologic agent of trichomoniasis - *Trichomonas gallinae* from the digestive track of a Rock Pigeon (*Columba livia*) (D. J. Forrester & Foster, 2008; Robert M Stabler, 1947). Although most cases of avian trichomoniasis are caused by *T. gallinae*, there are several other species associated with disease including *T.*

stablerli, T. gyptinella, a *T. vaginalis*-like sp, and many undescribed species or lineages (Gerhold et al., 2008).

Trichomonas is typically transmitted directly between birds, via mouth to mouth, or indirectly through consumption of infected feed or liquids (Andrea Bruno, Fedynich, Purple, et al., 2015; R. M. Kocan, 1969). Infections of *Trichomonas* varies by species of host with some developing subclinical infections and others developing severe disease with organ necrosis, caseation, and death (D. J. Forrester & Foster, 2008; R. M. Kocan & Herman, 1971). Trichomoniasis can lead to pathological changes in the upper digestive tract in the form of inflammation of the mucosa and caseous lesion, both of which could result in blockage of the esophagus and ultimately starvation (Andrea Bruno, Fedynich, Purple, et al., 2015; Grabensteiner et al., 2010). *T. gallinae* has been found to be able to infect Columbiformes, Falconiformes, Strigiformes, Passeriformes, Charadriiformes, Galliformes, Gruiformes, and Anseriformes (D. J. Forrester & Foster, 2008; R.M. Stabler, 1954; Robert M Stabler, 1947). There are no records of naturally occurring infections of *T. gallinae* in bobwhite, yet mortalities were noted when bobwhite were experimentally infected with *T. gallinae* (Andrea Bruno, Fedynich, Purple, et al., 2015; Levine et al., 1941). Bobwhite can potentially become infected by cofeeding on supplemental feed, that is meant to improve bobwhite habitat and survivability, with infected columbiforms (Andrea Bruno, Fedynich, Purple, et al., 2015; Henson et al., 2012; Morris et al., 2010). As bobwhite share geographic range and habitat with many columbiforms such as Mourning Doves (*Zenaida macroura*) and White Doves (*Zenaida asiatica*), both of which are hosts for *T. gallinae* and other *Trichomonas* spp., thus the risk of *Trichomonas* infections for bobwhite exists (Andrea Bruno, Fedynich, Purple, et al., 2015; Conti & Forrester, 1981).

Sarcocystis is a genus of protozoan parasite belonging to the family Sarcocystidae. These parasites form cysts in the muscle tissue of their hosts. Depending on the Sarcocystis species, the cysts may be microscopic or macroscopic (Erickson, 1940). Many species of *Sarcocystis* use avian species as an intermediate host and predators of the avian species as definitive host (Greiner, 2008; Odening, 1998). Sarcocystosis often presents as white cysts in the muscle tissue of birds, predominantly in the muscle tissues of the neck, tibia, and breast (Erickson, 1940; Kutkiene & Sruoga, 2004). Although sometimes being deeply embedded in the muscle tissue, the cysts tend to be benign (Lingappa et al., 2015). The small white cysts caused by *Sarcocystis* resemble that of a grain of rice embedded in the muscle tissue, giving rise to the common term 'Rice Breast' or 'Rice Breast Disease' for sarcocystosis (Szekeres et al., 2019). Sarcocystosis found in bobwhite are observed incidentally up necropsy or field dressing, with no indications that the infection has any major health impacts (Applegate et al., 2017).

Toxoplasma gondii, causative agent of toxoplasmosis, is a protozoan parasite with a world-wide distribution that infects virtually all warm blood animals including avian species and humans (Casagrande et al., 2015; Desmonts & Remington, 1980; Dubey, 2008; Dubey & Beattie, 1988; Dubey & Odening, 2001). *T. gondii* uses wild and domestic felids as definitive hosts and all warm-blooded animals including felids as intermediate hosts (Dubey, 2008, 2009; Dubey & Odening, 2001; Hill & Dubey, 2002). *T. gondii* was first discovered in a Tunisian rodent (*Ctenodactylus gundi*) in 1909 by Nicolle and Mancaux (Dubey, 2002, 2008). It wasn't until 1970 that the full life cycle of *T. gondii* was described with felids being the only definitive hosts (Dubey, 2008; Dubey & Beattie, 1988). In 1911, *T. gondii* was first discovered in birds, a Rock Pigeon in Sao Paulo, Brazil (Dubey, 2002, 2008).

One of the most well-known effects of toxoplasmosis aside from death, is the reported behavioral change of some species or individuals of intermediate host that make them more susceptible to predation (Dubey, 2009; Vyas & Sapolsky, 2010). An example would be rodents infected with *T. gondii* exhibit less fear and alertness in the presence of felids and other predators, which helps *T. gondii* complete its life cycle (Vyas and Sapolsky, 2010). Avian hosts can experience asymptomatic infections, but can also experience symptoms such as anorexia, depression, dull ruffled feathers, diarrhea, dyspnea, and death (Casagrande et al., 2015; Dubey, 2002, 2008; Dubey et al., 1993). In rare cases involving Island Canaries (*Serinus canaria)* cataracts and blindness has occurred (Dubey, 2008; Vickers et al., 1992). *T. gondii* is spread by felids dispersing oocysts in their feces (Dubey, 2008, 2009; Dubey & Beattie, 1988). These oocysts can survive in most environments for long periods of time, but under ideal conditions (moist soil) the oocysts can survive for months, and in some cases for years (Dubey, 2002; Dubey et al., 2020; Dubey & Beattie, 1988; Hill & Dubey, 2002). *T. gondii* oocysts can also be mechanically spread by flies, cockroaches, dung beetles, and earthworms (Dubey, 2008). Avian species can become infected with *T. gondii* by consuming oocysts from the environment or ingesting tissues infected with the parasite. Ground feeding birds are the most likely to get infected by ingesting oocysts from contaminated soil or arthropods (Dubey, 2008). Valley Quail (*Callipepla californica*) have had observed mortalities associated with toxoplasmosis and bobwhite dying after being experimentally infected with *T. gondii.* (Casagrande et al., 2015; Dubey et al., 1993). While avian species can be a for transmission route of *T. gondii* to humans, it is possible and cooking meat thoroughly is recommended to avoid potential exposure (Dubey, 2008). Toxoplasmosis is not attributed with large scale mortalities of wild birds, but it can pose a threat to small populations of naïve critically endangered species, such as the Hawaiian Crow that lost nearly 20% of their population to toxoplasmosis (Dubey, 2008; Work et al., 2000, 2002).

Bacterial Pathogens

Ulcerative enteritis is a disease caused by the bacterium *Clostridium colinum* (Bano et al., 2008; Beltran-Alcrudo et al., 2008; G. A. Berkhoff et al., 1974; H. A. Berkhoff, 1975, 1985; Cooper et al., 2013). Ulcerative enteritis can present as an acute and chronic disease in game birds, such as grouse (subfamily Tetraonini), bobwhite quail, partridge (subfamily Perdicinae), turkey (genus *Meleagris*) and pheasants (subfamily Phasianinae) (Bano et al., 2008; G. A. Berkhoff et al., 1974; Prescott, 2016). This disease also occasionally afflicts young chickens (*Gallus gallus*), pigeons (genus *Columba*), and American robins (*Turdus migratorius*) (Beltran-Alcrudo et al., 2008; Cooper et al., 2013). First reported in 1907, it was later described and named in 1974 by H.A Berkoff, ulcerative enteritis was primarily observed in quail which lead to the common name being 'Quail Disease' (Beltran-Alcrudo et al., 2008; H. A. Berkhoff, 1975; Prescott, 2016). This disease was noted as of epidemic proportion in bobwhite (Bano et al., 2008; H. A. Berkhoff, 1975). With a worldwide distribution, ulcerative enteritis risks afflicting all free-ranging and captive game birds across the globe (Beltran-Alcrudo et al., 2008).

Ulcerative enteritis is often transmitted via the fecal-oral route through the consumption of contaminated feed, water, or litter (Beltran-Alcrudo et al., 2008; Cooper et al., 2013; Prescott, 2016). With an incubation period of 3 days or less, symptoms start to present and peak mortality for the entire flock is 5-14 days after infection (Cooper et al., 2013). With close living quarters or communal food/drinking sources, mortality for the flock can reach 100% (G. A. Berkhoff et al., 1974; Cooper et al., 2013). The bacterium can also persist in the soil for extended periods, which in some cases results in annual reemergence of infections and subsequent mortalities. Clinical signs of infection include depression, reluctance to move, diarrhea, decreased appetite, huddling, dehydration, and death (H. A. Berkhoff, 1985; Cooper et al., 2013; Prescott, 2016). Clinical symptoms are sometimes missed as birds can die within 1-2 hours of onset of symptoms (Cooper et al., 2013). Gross lesions include hemorrhagic enteritis of the duodenum, yellow ulcers surrounded by hemorrhaging anywhere along the small and large intestines resulting in perforation of the gastro-intestine tract. Necrotic lesions and discoloration is often observed on the liver and spleen (Cooper et al., 2013; Wages, 1997). Ulcerative enteritis can have devastating effects on farmed and wild bobwhites (Beltran-Alcrudo et al., 2008).

Pasteurella multocida is an encapsulated bacterium that causes severe disease in avian and mammalian species. The disease caused by *Pasteurella multocida* is called pasteurellosis, but in bird species it is commonly referred to as Fowl or Avian Cholera (Botzler, 1991; Harper et al., 2006; F. Kellogg & Calpin, 1971). *P. multocida* was described and identified as the causative agent of avian cholera by Louis Pasteur in 1881 (Harper et al., 2006). Avian cholera-like disease was reported in domestic birds in Italy as early as the 1600's (M. D. Samuel et al., 2007). *P. multocida* is not limited to avian species, but can cause bovine hemorrhagic septicemia, enzootic pneumonia, and swine atrophic rhinitis (Botzler, 1991; De Alwis, 1992; Harper et al., 2006). Avian Cholera can infect over 180 species of free ranging birds and is considered to be one of the most impactful diseases to wildfowl in North America (Botzler, 1991; Harper et al., 2006). Nearly all avian species are susceptible to avian cholera under the right circumstances, with the exception of no reports of avian cholera among vultures (Botzler, 1991).

This bacterium can be transmitted by numerous routes but is most often transmitted by ingestion of a contaminated water source. Avian cholera is also transmitted by ingestion of bacteria through contaminated water sources, aerosol transmission in contaminated environments

(Harper et al., 2006; H. Li et al., 2019; M. D. Samuel et al., 2007). Wetlands are contaminated from dead or diseased birds with avian cholera, putting susceptible birds at risk (Harper et al., 2006; M. D. Samuel et al., 2007). In the last few decades, more than 50,000 birds have died to avian cholera in the United States alone (M. D. Samuel et al., 2007). While more common in waterfowl (Anseriforms), avian cholera is dangerous to young bobwhite (Bermudez et al., 1991; Dabbert et al., 1997; Derieux, 1983; Silvy et al., 1998). A flock of 5,000 farmed 6-week-old bobwhites underwent a mass mortality event of over 50% in 2 days, by day 6 mortality of the initial 5,000 bobwhite flock was 99% (Bermudez et al., 1991). It was found that deceased bobwhite from the flock were infected with *P. multocida*. The *P. multocida* isolated from the deceased bobwhite flock was then experimentally inoculated into healthy bobwhite, of which resulted in 50% mortality within 9 to 24 hours of infection (Bermudez et al., 1991). *P. multocida* (avian cholera) causes pre-acute disease with high rates of mortality (Bermudez et al., 1991; Derieux, 1983; H. Li et al., 2019).

Mycoplasma gallisepticum is a bacterium that causes disease in select avian species (Ferguson-Noel et al., 2017; Luttrell & Fischer, 2007; Tabler, 2020). The genus *Mycoplasma* contains over 100 species of bacterium that infects animals, humans, insects, and plants (Ferguson-Noel et al., 2017; Luttrell & Fischer, 2007; Razin et al., 1998; Razin & Hayflick, 2010). Mycoplasmosis is the infection of *Mycoplasma* sp., including *M. gallisepticum*, resulting in respiratory disease (Ferguson-Noel et al., 2017; Luttrell & Fischer, 2007; Nascimento et al., 2005). Avian mycoplasmosis was first described in 1926 in Turkey and chickens in 1936, later the etiologic agent would be determined to be *M. gallisepticum* (Nascimento et al., 2005). *M. gallisepticum* is considered the most economically and impactful mycoplasmal pathogen in turkeys and chickens (Ferguson-Noel et al., 2017; Luttrell & Fischer, 2007; Tabler, 2020).

M. gallisepticum can produce a variety of symptoms, including conjunctivitis, peri-orbital swelling, coughing, nasal discharge, respiratory rales, and infraorbital sinusitis (Ferguson-Noel et al., 2017; Luttrell & Fischer, 2007; Tabler, 2020). While *M. gallispecticum* is rarely noted to be considered pathogen resulting in mortalities, the secondary infections, such as infectious bronchitis, that can develop at the same time tend to be more impactful on host health (Ferguson-Noel et al., 2017; Luttrell & Fischer, 2007; Nascimento et al., 2005; Razin & Hayflick, 2010; Tabler, 2020). Young birds tend to be more susceptible to infections of *M. gallisepticum*, with the disease being worse in the winter months when environmental conditions are cold and stressful on the host (Dabbert et al., 1997; Ferguson-Noel et al., 2017; Tabler, 2020). *M. gallisepticum* has been detected in wild bobwhite, while often not attributed to mortalities, but it could potentially be a contributing factor to morbidity (F. Kellogg & Calpin, 1971; Madden et al., 1967; Razin & Hayflick, 2010; Stallknecht et al., 1982).

Viral Pathogens

West Nile Virus is a primarily vector-borne arbovirus, belonging to family of *Flaviviridae* and genus *Flavivirus*, that uses select species of birds as a definitive host (Allison et al., 2004; Colpitts et al., 2012; Lanteri et al., 2011; O'Donnell & Travis, 2007; Petersen et al., 2013). West Nile virus is in the Japanese Encephalitis serocomplex, which also includes Japanese encephalitis virus and St. Louis encephalitis virus (Colpitts et al., 2012; Lanteri et al., 2011; O'Donnell & Travis, 2007; Petersen et al., 2013). Approximately 60 species of mosquitos can transmit West Nile Virus in the United States, although main vector species include *Culex pipens*, *Culex tarsalis*, and *Culex quinquefasciatus* (Colpitts et al., 2012; Lanteri et al., 2011; O'Donnell & Travis, 2007; Pérez-Ramírez et al., 2014; Phipps et al., 2007). West Nile virus depends on a passerine-*Culex* cycle to maintain infected reservoir hosts and vectors (Lanteri et al., 2011; Petersen et al., 2013). The first identifiable case of West Nile virus was detected from a febrile patient in the West Nile district in Northern Uganda in 1937 (O'Donnell & Travis, 2007; Petersen et al., 2013; Sejvar, 2003). West Nile virus was first detected in North America (New York City, New York) in 1991. Since then, West Nile virus has spread south and west across the United States. In the 8 years between being first detected in the United States, over 20,000 human cases and over 24,000 equine cases of WNV infection were reported (O'Donnell & Travis, 2007; Petersen et al., 2013). Nearly 75% of infected humans are asymptomatic; the other 25% of infected humans experience flu-like symptoms including headache, nausea, muscle soreness, swollen lymph glands, and fever (Mitchell et al., 2018; O'Donnell & Travis, 2007). In less than 1% of cases, those infected will develop West Nile Encephalitis or Meningitis, which can result in coma, tremors, paralysis, and death (Mitchell et al., 2018; O'Donnell & Travis, 2007). Humans and most mammals only develop low-level serum viremia, thus are dead-end hosts for West Nile virus (O'Donnell & Travis, 2007; Petersen et al., 2013).

Avian species infected with West Nile virus can experience a range of symptoms, some avian species are completely asymptomatic, while others can develop loss of coordination, head tilt, tremors, weakness, lethargy, and death (Lanteri et al., 2011; O'Donnell & Travis, 2007; Phipps et al., 2007). Age is considered to be a factor in West Nile virus infection, as chicks and juveniles have less feathers, defensive behaviors to arthropod vectors, and tend to be more stationary than adults (Blackmore & Dow, 1958; Kale et al., 1972; McLean et al., 1989; Pérez-Ramírez et al., 2014). Hematophagous ectoparasites such as the Cliff Swallow Bug (*Oeciacus vicarius*), have had West Nile virus isolated from them, but thus far have been unable to vector West Nile virus experimentally (Lwande et al., 2013; Pérez-Ramírez et al., 2014). However, West Nile Virus can be experimentally transmitted by some ticks (Abbassy et al., 1993;

Hutcheson et al., 2005; Pérez-Ramírez et al., 2014). Birds belonging to the family Corvidae are particularly susceptible to infection of West Nile virus and often die (Allison et al., 2004; Center for Disease Control, 2016; Gibbs et al., 2005; Komar et al., 2003). Bobwhite can be infected with West Nile virus, some of which resulted in mortality (Center for Disease Control, 2016; Pérez-Ramírez et al., 2014; Pezzin et al., 2016). Due to low viremia, the bobwhite is not considered a reservoir host for West Nile virus (Center for Disease Control, 2016; Pérez-Ramírez et al., 2014).

Reticuloendothelosis Viruses (REVs) are a small group of avian retroviruses, belonging to the family *Retroviridae*, that have immunosuppressive and sometimes oncogenic effects in various bird species including Anseriformes, Galliformes, and Passeriformes (Caleiro et al., 2020; Ferro et al., 2017; Liss & Bose, 2008; Stewart et al., 2019). Reticuloendotheliosis was initially identified in a domestic turkey in 1958 and occurs in turkey species throughout the world (Drew, 2007). REV's rapidly induces a severe immunosuppressed state in infected birds, leading to further disease associated with REV. Diseases induced by REV include visceral reticuloendotheliosis, spleen necrosis, hepatomegaly, splenomegaly, thymic and bursal atrophy, lymphoid nerve lesions, anemia, runting, and abnormal feather development (Barbosa et al., 2006; Hrdlickova et al., 1999; Liss & Bose, 2008). Transmission of REVs occurs vertically and horizontally. Vertical transmission of REVs occurs when the mother releases viruses, contaminating the egg during development (Hrdlickova et al., 1999). Horizontal transmission of REVs occurs from bird-to-bird contact, though some insect species may play a role (Hrdlickova et al., 1999; Liss & Bose, 2008).

Reticuloendotheliosis viruses are more often found in Wild Turkeys (*Meleagris gallopavo*) and domestic chickens (*Gallus gallus*) than other avian species (Caleiro et al., 2020; Stewart et al., 2019). Although rare, REVs are considered as risk some endangered species, such as the Attwater's Prairie chicken (*Tympanuchus cupido attwateri*), because REVs can infect and negatively impact related species such as the Greater Prairie Chicken (*T. cupido*), the Japanese Quail (*Corurnix coturnix japonica*), and Northern Bobwhite Quail (Barbosa et al., 2006; Drew, 2007; Hopkins, 2018).

Avian adenoviruses are a ubiquitous virus found in domestic and wild birds throughout the world (DuBose, 1967; Fitzgerald, 2007; S. Jack et al., 1994). Avian adenoviruses belong to the family Aviadenoviridae and consist of 5 species (fowl adenovirus A through E with 12 serotypes) (DuBose, 1967; Fitzgerald, 2007; Singh et al., 2016). The first *aviadenovirus* was isolated from a chicken embryo in 1957, the virus ultimately caused the death of the embryo. Aviadenovirus have be identified and isolated from chickens, turkeys, Guinea fowl (family *Numididae*), kestrels, bobwhite, Gambiel's quail (*Callipepa gambelii*), pigeons, common ostriches (*Struthio camelus*), and various psittacine birds, including Budgerigars (*Melopsittacus undulatus*), lovebirds (*Agapornis* sp.), Cockatiels (*Nymphicus hollandicus*), cockatoos (*Cacatua* sp.), conures, macaws, parrots, and parakeets (Fitzgerald, 2007).

Quail bronchitis is caused by quail bronchitis virus (avian adenovirus A serotype 1), and is characteristically a highly contagious and acute respiratory disease of bobwhite (Barnes, 1987; DuBose, 1966, 1967; Fitzgerald, 2007; S. W. Jack & Reed, 1988). Quail bronchitis was first described and isolated in 1949 by Olson in a bobwhite in West Virginia suffering for respiratory disease (DuBose, 1967; Fitzgerald, 2007; S. W. Jack & Reed, 1988). Quail bronchitis is transmitted through bird-to-bird contact, airborne transmission, and mechanical transmission (DuBose, 1966, 1967; Fitzgerald, 2007). Clinical symptoms of quail bronchitis include depression, isolation from other quail, naso-ocular discharge, open mouth breathing, wing droop, and increased respiratory sounds. Lesions associated with quail bronchitis infection include lesions in the trachea with opaque spots present, reddening and consolidation of lung surrounding bronchial hilus (DuBose, 1967; S. Jack et al., 1994; S. Jack & Reed, 1990; S. W. Jack & Reed, 1988). Being highly contagious, quail bronchitis can spread through a large captive bobwhite flock in 10 days, and wild quail coveys in even less time (DuBose, 1967; Fitzgerald, 2007; S. Jack & Reed, 1990; S. W. Jack & Reed, 1988). With an incubation period of 2-4 days, signs of infection develop quickly with mortalities following soon after (DuBose, 1967; Fitzgerald, 2007; S. Jack & Reed, 1990; S. W. Jack & Reed, 1988). Young bobwhite are especially susceptible to quail bronchitis disease, with wild and experimental observations recording a minimum of 50% mortalities of bobwhite under 4 weeks old and mortalities reaching 100% of bobwhite under 4 weeks being a common outcome (S. Jack et al., 1994; S. Jack & Reed, 1990; S. W. Jack & Reed, 1988). Quail bronchitis is a major risk of depopulating young bobwhite in wild coveys and captive flocks. There is no current treatment for quail bronchitis, and the only management strategy for the disease is to limit the spread by not overcrowding captive bobwhite operations (S. W. Jack & Reed, 1988).

External Parasites

Ticks

Avian species play a varied role in the ecology of ticks as some avian species prey on ticks for food and some tick species are dependent on avian species as hosts to feed and complete their lifecycles (Brinkerhoff et al., 2019; Teel et al., 2010). Avian hosts are particularly important hosts because while feeding on birds, ticks can be transported great distances, which greatly increases their ability to expand their range. One important example would be the invasive Asian longhorned tick (*Haemaphysalis longicornis*). One theory of H. longicornis' rapid spread westward across the United States from New Jersey, is being transported great distances via wildlife hosts, including birds (Brinkerhoff et al., 2019; Choi et al., 2014; Thompson et al., 2020; Tufts et al., 2021). Aside from being essential to some tick species life cycles and transporting ticks, birds are also subject to being infected and even acting as a reservoir for some tick-borne diseases. Transmission experiments revealed that certain North American avian species can be competent reservoirs for the Lyme Disease agent (*Borrelia burgdorfei*) e.g. the American Robin (Becker & Han, 2021; Brinkerhoff et al., 2019; Richter et al., 2000)). To find hosts, ticks detect carbon dioxide and other chemical cues while questing, making nesting and ground dwelling avian species easy hosts to detect and infest (Brinkerhoff et al., 2019). While a frequent parasite found on bobwhite, it is believed that bobwhite contribute a considerable pressure on tick populations through predation, which in turn reduces the number of tick-borne diseases in the environment (Patterson & Knapp, 2018).

Amblyomma maculatum is a hard tick belonging to the family Ixodidae (Lado et al., 2018; Teel et al., 2010). Commonly referred to as the Gulf Coast tick, *A. maculatum* was first described by Koch in 1844 (Lado et al., 2018). The Gulf Coast tick is an arthropod of medical, veterinary, and economic importance. Several zoonotic pathogens such as *Rickettsia parkeri* and Panola Mountain *Ehrlichia*, have be isolated from Gulf Coast ticks (Teel et al., 2010). The Gulf Coast tick is the primary vector of *Hepatozoon americanum*, the etiological agent of American canine hepatozoonosis and it can cause ascending tick paralysis in dogs and 'gotcha ear' on ruminants (Ewing & Panciera, 2003; Gothe et al., 1979; Parker et al., 1939).

Both larval and nymphal life stages of *A. maculatum* use avian species as hosts. Approximately 61% of larval and 50% of nymphal *A. maculatum* use avian species as hosts (Teel et al., 2010). Bobwhite are among the preferred hosts for larval and nymphal *A. maculatum*, this is attributed to accessibility as bobwhite are ground dwelling birds and that engorgement time for *A. maculatum* attached to bobwhite is significantly shorter than compared to other potential hosts (Koch & Hair, 1975; Teel et al., 2010). Despite being an easily accessible and preferred host for *A. maculatum*, there hasn't been any deleterious effects associated with Gulf Coast tick parasitism for bobwhite (Teel et al., 2010; Willaims & Hair, 1976).

Amblyomma americanum is a hard tick belonging to the family Ixodidae (Barrett et al., 2015; Scott Dahlgren et al., 2016). This tick is known as the Lone Star tick due to the distinctive single white spot on the scutum of adult females. This tick was first described in 1758, is widely distributed in the Eastern United States, and uses a diverse list of host species including birds, mammals, and reptiles (Kierans & Lacombe, 1998). *A. americanum* is a competent vector of *Rickettsia* spp., *Ehrlichia* spp., tularemia, Heartland virus, and *Cytauxzoon felis* (Barrett et al., 2015; Egizi et al., 2020; Paddock & Yabsley, 2007). The most important tick-borne disease associated with *A. americanum* is *Rickettsia rickettsia*, the causative agent of Rocky Mountain Spotted Fever (Egizi et al., 2020; Scott Dahlgren et al., 2016). Left untreated for too long, Rocky Mountain Spotted Fever can result in disseminated intravascular coagulopathy and multi-system organ failure, which can lead to permanent disabilities or death in humans (Scott Dahlgren et al., 2016). Another disease of notability is galactose-α-1,3-galactose (Alpha-gal) syndrome, which is a red meat allergy associated with tick bites (Crispell et al., 2019). The severity of the red meat allergy ranges from being very minor gastrointestinal distress to anaphylactic shock and death (Crispell et al., 2019).

A. americanum is commonly found on ground dwelling species of bird, including bobwhites, turkeys, and pheasants (Harmon et al., 2015). Although an important vector of numerous pathogens of humans and animals, *A. americanum* isn't known to cause significant effects on bobwhite health (Bolte et al., 1970; Doster et al., 1980; Yabsley et al., 2005).

Haemaphysalis leporispalustris is a hard tick belonging to the family Ixodidae (Kollars & Oliver, 2003; Labruna et al., 2000; Labruna & Leite, 1997). Being first described in 1869 by Packard, *H. leporispalustris* has been noted to have a range extending from Alaska to Argentina (Labruna et al., 2000; Labruna & Leite, 1997). In North America, the primary host for *H. leporispalustris*, often called the rabbit tick, is wild rabbits (*Sylvilagus* spp.) and hares (*Lepus* spp.) (Kollars & Oliver, 2003). Rabbits and hares are able to host *H. leporispalustris* for all its lifecycle stages. All three stages are commonly found on lagomorphs, but only nymphal and larval stages are found on avian hosts (Kollars & Oliver, 2003).

H. leporispalustris is the most common tick species found on bobwhite (Doster et al., 1980). Infestations of *H. leporispalustris* are generally light to moderate on adult birds, but young bobwhite can have heavier burdens. Some young bobwhite with heavy burdens of *H. leporispalustris* have been reported to be emaciated and individual mortalities have been attributed to gross infestations (Bergstrand & Klimstra, 1964). *H. leporispalustris* can transmit *Fransicella tularensis* (causative agent of tularemia which is a fatal disease of humans) to bobwhite, which in one case was the source of human infection (Barnes, 1987; F. Kellogg & Calpin, 1971).

<u>Chewing Lice</u>

Mallophaga, known as chewing lice, are apterous insects and the most prevalent external parasites found on birds (Bergstrand & Klimstra, 1964; Doster et al., 1980; Richards & Davies, 1977). Most Mallophaga (e.g., *Gonoides orygis* and *Oxyplipeurus clavatus*) feed on fragments of feathers, hair and other epidermal products. Some Mallophaga (e.g., *Menacanthus* sp.) feed on blood and feather fragments (Richards & Davies, 1977). Most infestations of Mallophaga are asymptomatic and disease (loss of plumage and deterioration in condition) is only noted with heavy burdens (Richards & Davies, 1977). Once the host dies, mallophagan must relocate to a living host with most species only having a few hours to a few days to find a new host. There have been instances of Mallophaga clinging to Culicid and Hippoboscid flies in order to find a new host (Richards & Davies, 1977).

The common species of Mallophaga on bobwhite include *G. ortygis*, *O. clavatus*, and *Menacanthus* sp. (Bergstrand & Klimstra, 1964; Doster et al., 1980; F. Kellogg & Calpin, 1971; F. E. Kellogg & Doster, 1972; Richards & Davies, 1977). The effects of the Mallophaga on bobwhite are considered minimal, as infestations haven't been associated with disease (Bergstrand & Klimstra, 1964; Richards & Davies, 1977).

Internal Parasites

<u>Nematodes</u>

Nematodes (roundworms) make up the phylum Nematoda. Often associated with parasitic infections, nematodes inhabit a variety of hosts including mammals, birds, reptiles, amphibians, fish, insects, and even plants (Kiontke & Fitch, 2013). Nematodes are easily described as a tube inside a tube, as their structure is that of a non-segmented worm (Kiontke & Fitch, 2013). Nematodes can be found in the deep sea, deserts, polar permafrost, and everywhere in between (Prosser et al., 2013). Among some of the most well-known nematodes are the Human Guinea worm (*Dracunculus medinensis*) that grows up to 3 feet long inside human hosts in Africa and the Raccoon Roundworm (*Baylisascaris procyonis*) that can be fatal in humans (Cleveland et al., 2018; Sapp et al., 2017). In avian species, nematodes infect nearly every species in every part of the world. The sites infected in the host depends on the species of nematode; some infect the intestines and ceca of birds (e.g., *Heterakis gallinarum* and *Aulonocephalus pennula*), the proventriculus (*Tetrameres* sp.), the muscle tissues (*Physaloptera* sp.), and the eyes (*Oxyspirura petrowi*) (A. Bruno et al., 2019; Henry et al., 2017; Olsen & Fedynich, 2016). The health implications associated with nematode infestations varies from asymptomatic to lethal depending on the species of nematodes, where in the host they are, and the parasitic burden (A. Bruno et al., 2019; Kiontke & Fitch, 2013)).

Heterakis gallinarum is a parasitic nematode, belonging the Heterakidae family, that thrives in the ceca of gallinaecaous birds (Cupo & Beckstead, 2019; Fedynich, 2008). *H. gallinarum* has a direct lifecycle that does not require an intermediate host, meaning that birds in high densities, whether living in the wild or raised in captivity, are at the high risk for infection (Fedynich, 2008; A. A. Kocan et al., 1979). Eggs of *H. gallinarum* are directly passed from the feces of an infected bird and infect a non-infected bird upon consumption (Cupo & Beckstead, 2019; Fedynich, 2008).

Most infestations of *H. gallinarum* go unnoticed, as the average infection has little to no indicators of negative health impacts (Cupo & Beckstead, 2019; Fedynich, 2008). In severe infestations with heavy burdens, the host can experience weight loss, petechial hemorrhages, bloody exudate overlying the mucosa in the ceca (Cupo & Beckstead, 2019). While the nematodes themselves don't often cause health detriments, they do vector a protozoon parasite called *Histomonas meleagridis*. *H. meleagridis* is the causative agent of Histomonosis as known as Blackhead disease (Lund & Chute, 1971). Blackhead disease is characterized by severe ulceration of the ceca, secondary involvement of the liver, and mortalities in most avian species. In turkeys, grouse, and chickens blackhead disease is often severe and potentially fatal (Cupo & Beckstead, 2019). *H. gallinarum* have been found in wild bobwhite and *Hi. meleagridis* has also been isolated from wild bobwhite, but with both present the infections seemed mild. Bobwhite experimentally infected with *Hi. meleagridis* fared better than grouse, chicken, and turkeys and suffered limited health impacts and no mortalities (Lund & Chute, 1971).

Aulonocephalus pennula is a parasitic nematode, belonging to the Subuluridae family, that infects the ceca, large and small intestine of galliforms. First described in 1935 by Chandler, *A. pennula* was largely considered a negligible parasite with little to no impact on its host, thus its life cycle is relatively unknown (Nicholas R. Dunham, Henry, et al., 2017). Recently, there is considerable interest in *A. pennula* as a possible factor to the declining Oklahoma and Texas populations of bobwhite (Blanchard et al., 2019; Nicholas R. Dunham, Henry, et al., 2017; Nicholas R. Dunham, Peper, et al., 2017). *A. pennula* is found in the ceca and small/large intestine and is thought to interfere with nutrient and water absorption of bobwhite (Nicholas R. Dunham, Henry, et al., 2017). Pathological effects are seen when the burden of *A. pennula* exceeds that of 200 worms. Pathological consequences of heavy burdens of *A. pennula* include weight loss, dehydration, and even death through malnutrition. These effects as a result of *A. pennula* infestation are compounded during months of drought or during times of food scarcity such as winter (Nicholas R. Dunham, Henry, et al., 2017; Nicholas R. Dunham, Peper, et al., 2017). Studies have shown an increase of burdens of *A. pennula* during the winter months, which has negative impacts on bobwhite fat stores, thus making overwintering much more difficult to survive (Blanchard et al., 2019; Brym et al., 2018b; Nicholas R. Dunham, Peper, et al., 2017; Rogers, 1987).

Tetrameres sp. are a parasitic nematode, belonging to the family Tetrameridae, that infects the proventriculus of avian species. Within the family Tetrameridae, there are three genera; *Tetrameres, Microtetrameres,* and *Geopititia* which are extremely difficult to distinguish morphologically as generally only females are found and they have few diagnostic characteristics. *Tetrameres* was first described by Diesing in 1835, with the first lesion associated with *Tetrameres* described in 1908 by Rust in Germany. Clinical signs of *Tetrameres* infections include weakness, loss of appetite, diarrhea, emaciation with atrophy of thoracic muscles. In cases with high burdens of *Tetrameres*, swelling of the proventriculus and necrosis of the swollen tissues can occur. *Tetrameres pattersoni* is the most common species reported in bobwhite. Infected bobwhite can experience the clinical symptoms listed above and high prevalences (e.g., 70%) and burdens (as high as 65) have been reported. No correlation between *T. pattersoni* infections and age of infected bobwhite has been reported. So far, no bobwhite deaths have been directly attributed to *T. pattersoni* infections.

Physaloptera are a group of nematodes in the family Physalopteridae, that infects the stomach or small intestine of a variety of definitive mammalian, avian, reptile, and amphibians hosts (Kalyanasundaram et al., 2017; Morgan, 1943). *Physaloptera* was first described in 1819 by Rudolphi, and later was first described infecting an avian species in 1829 by Creplin from a shrike (*Lanius minor*) (Morgan, 1943). In the definitive host, *Physaloptera* spp. attach to the walls of the duodenum and stomach, which may result in gastritis, gastrointestinal upset, erosion of the mucosa, ulcers, and vomiting (Kalyanasundaram et al., 2018). Numerous invertebrates and vertebrates, including birds, can serve as intermediate or paratenic hosts for *Physaloptera* spp. (Kalyanasundaram et al., 2017, 2018). In paratenic and intermediate hosts, *Physaloptera* sp. larva encysts in the muscle tissue where they remain until an appropriate definite host ingests the infected animal.

There have been several reports of *Physaloptera* sp. larvae in the muscles of bobwhite, but prevalence tends to be low (7-8%) (Kalyanasundaram et al., 2017, 2018). The pathological impacts are presumed to be limited, but there are few data on effects of encysted *Physaloptera* in the breast muscle, it is theorized that *Physaloptera* sp infections result in parasite-induce immunosuppression (Cox, 2001; Kalyanasundaram et al., 2018). It is most often accepted that *Physaloptera* sp. infections have limited effects on bobwhite health (Williams et al., 2018). In addition, in years with higher *Physaloptera* prevalence in bobwhite, notable decreases in bobwhite populations were recorded in Texas (Kalyanasundaram et al., 2018). The definitive host of the Physaloptera in bobwhite is unknown but a study in Texas found that cytochrome oxidease I gene sequences had an 87% identity to *P. retusa*, and an 86% molecular identity match to *P. turgida* (Kalyanasundaram et al., 2018). This data confirmed the parasite is a *Physaloptera* sp. but did not help identify the definitive host.

The eyeworm, *Oxyspirura petrowi,* is a heteroxenous nematode, belonging to the Thelaziidae, that infected birds across the United States and has been suggested as a significant pathogen of bobwhite in Texas (Blanchard et al., 2019; Nicholas R. Dunham, Bruno, et al., 2016). First described in 1929 by Skrjabin, *O. petrowi* was first recovered from grouse in Germany. Although *O. petrowi* is most commonly reported in the United States, it has also been reported in Czechoslovakia and Iraq (N. R. Dunham & Kendall, 2017). *O. petrowi* inhabits the ocular tissues of their numerous gallinaceous bird hosts. Because it is commonly reported in bobwhite, it is often referred to as the Quail eyeworm. Crickets and grasshoppers are confirmed intermediate hosts (Blanchard et al., 2019; Kistler et al., 2016).

O. petrowi is suspected as a factor in the ongoing population decline of bobwhite, at least in Texas, despite previously being considered a parasite of little importance (Nicholas R. Dunham et al., 2014). In the Rolling Plains eco-region, surveys have found prevalences ranging from 41% - 97% (Brym et al., 2018a; Nicholas R. Dunham et al., 2014; Nicholas R. Dunham, Bruno, et al., 2016). Some bobwhite can harbor up to 69 eyeworms (Brym et al., 2018a; N. R.

Dunham & Kendall, 2017; Nicholas R. Dunham, Reed, et al., 2016). *O*. petrowi infections are reported to cause inflammation, edema, and cellular damage to the eye of infected bobwhite (N. R. Dunham & Kendall, 2017; Villarreal et al., 2012). Further studies have highlighted atrophy and lesions of the Harderian gland, located behind the bobwhite's eyes, and corneal erosion associated with *O. petrowi* infections (Nicholas R. Dunham, Reed, et al., 2016). It is thought that this damage to the eyes could lead to blindness of bobwhite, increasing morbidity (Andrea Bruno, Fedynich, Smith-Herron, et al., 2015; Nicholas R. Dunham, Bruno, et al., 2016; Nicholas R. Dunham, Reed, et al., 2016). Studies into the extent of the impact *O. petrowi* has on bobwhite populations is ongoing. Current management strategies involving medicated feed (QuailGuard®) and specialized feeders (Quail Safe Feeder ©) are being developed to combat *O. petrowi* infections.

Cestodes

Cestodes (or tapeworms) are extremely common parasites that infects mammals, birds, reptiles, amphibians, fish, and insects. A distinguishing characteristic of cestodes is their segmented bodies, with some species producing eggs in each segment (proglottid). Avian species have the most diverse cestode fauna of any vertebrate group. Of the 4,000 nominal species of cestodes, approximately 1,700 infect avian species (Mclaughlin, 2008). Most tapeworms in avian species live in the intestines, but some species may be located in the ceca or under the gizzard lining (Mclaughlin, 2008). Most avian infecting cestode species require at least one intermediate host species in their life cycles. Severe infections of cestodes can result in inflammation of the intestines, weakness, emaciation, diarrhea, changes in posture or locomotion, and feeding behaviors (Mclaughlin, 2008).

For bobwhites, cestodes are believed to only rarely cause mild intestinal obstruction and prevalences ranging between 1-14% (Davidson et al., 1982; Demarais et al., 1987; Herzog et al., 2021; Olsen et al., 2016). Despite the lack of reported mortalities, the reports of morbidities associated with cestode infestations, suggests these parasites should be further investigated.

Acanthocephala

Acanthocephala (thorny-headed worms) is a phylum of parasitic worms can be distinguished by their thorny proboscis (Richardson & Nickol, 2008). The >1,100 described species of Acanthocephala infects all classes of vertebrates as definitive hosts (Richardson & Nickol, 2008). Bony fish are the most parasitized group and reptiles being the least parasitized. There are few reported clinical signs of Acanthocephala infections in avian birds, but emaciation and stunted growth are rarely reported (Richardson & Nickol, 2008). Pathological impacts of Acanthocephala do not seem to be dependent on intensity of infections in most species, as low intensity infections may present pathology in hosts like lower body weight, and changes in basal metabolic rates and thermoregulations, while hosts with high intensity infections may not show any clinical signs of infection (Richardson & Nickol, 2008; Shea et al., 2020). It is thought that the extent of pathogenesis is more dependent on the on the nutritional status of the host rather than the intensity of the infestation (Richardson & Nickol, 2008).

The most common acanthocephalan in bobwhite is *Mediorhynchus papillosis*, although it is generally found in low prevalence (<11%) (A. Bruno et al., 2019; Herzog et al., 2021; Olsen et al., 2016; Olsen & Fedynich, 2016; Shea et al., 2020; Villarreal et al., 2016). Although not heavily investigated, *M. papillosis* thought to be able to induce emaciation, or alter the infected bobwhite's thermoregulation and energy conservation (Dabbert et al., 1997; Richardson & Nickol, 2008; Rogers, 1987).\

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CHAPTER 3

BASELINE HEALTH SURVEY OF NORTHERN BOBWHITE QUAIL (*COLINUS VIRGINIANUS*) FROM WESTERN OKLAHOMA

[1]Wyckoff, S.T., Judkin,Tell., Nemeth, N, Ruder, M.G., Martin, J., Kunkel, M., Adcock, K., Yabsley, M.J. 2021. To be submitted to the Journal of Wildlife Diseases

Abstract

The Northern Bobwhite (*Colinus virginianus*) has been undergoing a range wide population decline. To better understand the decline of bobwhite across Oklahoma and the possible causes, a health survey was conducted. In this study, we evaluated the health of free-ranging bobwhite from nine sites in western Oklahoma. From 2018-2020, 206 bobwhite were evaluated for gross and microscopic lesions and tested for select pathogens. In general, bobwhite were in overall good nutritional condition with ample muscle mass and fat stores. No significant gross lesions, including those consistent with wet or dry avian pox, avian influenza, gastroenteritis (e.g., clostridial), mycoplasmosis, or conjunctivitis, were observed. There was no evidence of infection with or exposure to reticuloendotheliosis virus, West Nile virus, respiratory *Mycoplasma* spp., *Pasteurella multocida*, intestinal *Eimeria*, blood parasites (*Haemoproteus, Leucocytozoon*, and *Plasmodium*) and oral *Trichomonas* spp. Two pathogens of potential concern were detected in tissues from bobwhite: *Toxoplasma gondii* (2.3% prevalence) and avian adenovirus (8.6% prevalence). *Tetrameres* (nematode) was an incidental finding in the proventriculus of six (3%) bobwhite. No significant histologic lesions were detected in a 20% subset of bobwhite. Two parasite species, *Physaloptera* sp. (12% prevalence, mean intensity 5.2 ± 7.3) and *Sarcocystis* sp. (1%, 1), were detected in the breast muscle. Low intraspecific genetic diversity was noted for the *Physaloptera* specimens and sequences were most similar to *Physaloptera* sequences from bobwhite in Texas. A low intensity of chewing louse (*Goniodes ortygis, Menacanthus* sp., and *Oxylipeurus clavatus*) and ticks (*Amblyomma americanum*, *Amblyomma maculatum*, and *Haemaphysalis leporispalustris*) was observed. A subset of bobwhite had non-detectable levels of select toxins and heavy metals in liver; a small number had low levels of iron, manganese, zinc, molybdenum, and copper, none of which were considered diagnostically relevant. In general, bobwhite from western Oklahoma appeared to be in good health with a low diversity of pathogens detected, but future work is needed to understand disease risks for this population.

INDEX WORDS: Population decline, avian adenovirus, parasites, pathogens, *Toxoplasma gondii*

Introduction

The Northern Bobwhite (bobwhite, *Colinus virginianus*), is one of the most sought after and influential game species in Oklahoma. The bobwhite has a high economic importance due to an increased demand as a delicacy food and for shooting-preserve operations (F. Kellogg & Calpin, 1971). Since the mid 1960's, the bobwhite population has undergone an ~85% decline throughout its historic range, averaging a ~4% decrease per year. In most regions, there has been a considerable reduction in density, with complete disappearance apparent in some areas (Sauer et al., 2017). Currently, Oklahoma has one of the most robust populations of bobwhite, resulting in considerable interest among hunters; however, even these populations have undergone recent declines (ODWC, 2017). The North American Bird Breeding Survey has provided population data on over 425 species of birds, including the bobwhite, for over 50 years using volunteer roadside surveys and rigorous protocols, allowing for the tracking of long-term population trends (Sauer et al., 2013, 2017). In Oklahoma, statewide roadside surveys are frequently employed to count bobwhite from vehicles during 20-mile-long routes (Janus, 2018; Judkins, 2020b). This population survey method is conducted twice a year in Oklahoma, and statewide roadside survey averages have fallen from ~11 bobwhite observed in 1990 to approximately ~1.5 in 2020 with a nearly 42% drop between 2019 and 2020 (Judkins, 2020b). The 2020 roadside quail survey results also were 1.68 bobwhite counted per 20-mile survey, which is 68% below the 31 year (1990-2020) average of 5.1 (Janus, 2018; Judkins, 2020b). 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, 2020a, 2020b). A concurrent study in western Oklahoma used collared radio transmitters and backpacked GPS units to track bobwhite to better understand their movements, habitat use, feeding behaviors, and to record mortalities. Thus far, research consistently leads to the conclusion that while the bobwhite population in Oklahoma is faring better than populations in other parts of their endemic range, it is experiencing the same overall trend of population decline (L. A. Brennan, 2006; Hernández et al., 2013).

Potential causes for the significant population decline of bobwhite across their historic range have been investigated for decades. The 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 bobwhite (L. A. Brennan & Kuvlesky, 2005; Doggett & Locher, 2018; Miller et al., 2019). Farming and energy extraction not only changes the habitat, possibly making it less favorable for foraging or nesting success or predator avoidance, but also can cause potential exposures to toxicants (e.g., pesticides and heavy metals), which can result in negative effects on wildlife health. In addition, several studies on parasites, diseases, and toxin exposure of bobwhite have been published (A. Bruno et al., 2019; Brym et al., 2018; Dunham, Peper, et al., 2017; King et al., 1981; Shea et al., 2020; Turaga et al., 2015; Wilson & Crawford, 1988). However, contemporary studies on free-ranging bobwhite quail have primarily focused on parasites or specific infectious agents (e.g., influenza virus, West Nile virus, intestinal microbiota) or were limited in geographic scope (Ferro et al., 2012; Su et al., 2014; Urban et al., 2013). Although there have been several studies conducted on the ecology of bobwhites in Oklahoma and the possible threats to bobwhite health (e.g., habitat, movements, and predation), there are little data on the health of bobwhite in Oklahoma. The purpose of this study was to establish baseline health survey data on bobwhite quail from across western Oklahoma by conducting gross and microscopic postmortem examinations and laboratory testing for select pathogens.

Materials and Methods

Study sites, animal capture, handling, and sampling

From 2018-2020, bobwhite quail were sampled from nine Wildlife Management Areas in the western region of Oklahoma (Figure 3.1). Each year, 24" x 24" x 8" funnel wire traps (Smith et al., 1981) were deployed during two 2-week sessions in early August and mid-October, for a total of 6 trapping sessions. Traps were pre-baited with a mixture of corn and seed two weeks to one month before the trapping period to increase capture success.

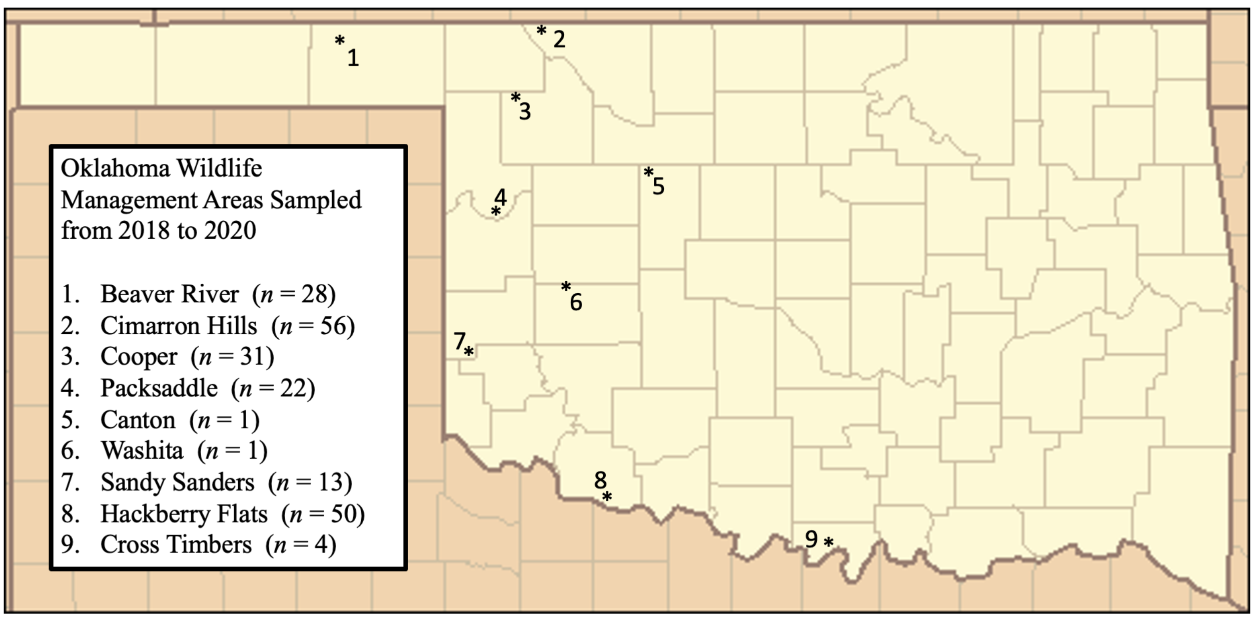

Figure 3.1 Wildlife management areas (WMA) in Oklahoma sampled and number of bobwhite quail (*Colinus virginianus*) sampled during 2018-2020.

Bobwhite were weighed in a cloth bag on a spring scale (the nearest 0.1 gram). Each quail was given a cursory external/physical examination to assess their condition. For a subset of quail, a blood sample was collected from the jugular vein using a 29 gauge, 1 ml insulin syringe (VetOne, Boise, ID). A portion of the whole blood was deposited into a serum separator tube

(Microtainer®, Franklin Lakes, NJ), and the remaining blood was applied to a Nobuto filter strip (Advantec, Japan). The serum separator tubes containing blood were centrifuged at 13,500 rpm (18,000 x g) for two minutes and frozen at -20 C until testing. Nobuto strips were placed in a 50 ml conical tube with a metal twist-tie to allow the blood saturated strips to dry before being stored in paper envelopes at ambient temperature to avoid the loss of wet blood samples through smearing. Bobwhite were euthanized with CO_2 inhalation followed by cervical dislocation and the carcasses were immediately frozen to -20 C until necropsy. All animal trapping and handling techniques were reviewed and approved by the University of Georgia Institutional Animal Care and Use Committee (A2018 04-001 and A2020 11-010).

Frozen quail carcasses were shipped to the Southeastern Cooperative Wildlife Disease Study (Athens, GA) for storage and necropsy. Age class initially was determined by examining the primary covert feathers for buffing as described (Koerth et al., 2011; Petrides & Nestler, 1943). A more accurate age subsequently was determined by counting and measuring primary feathers undergoing growth and molt (Petrides & Nestler, 1943). Sex was confirmed during necropsy by gross observation of gonads.

Bobwhite carcasses were carefully examined for ectoparasites, which were removed and preserved in 70% ethanol until identification (Doster et al., 1980). Ticks were identified to species using dichotomous keys (Coley et al., 2015; Hoskins & Cupp, 1988; Keirans & Durden, 1998; Levin et al., 1997; Martins et al., 2010). Chewing lice were identified using dichotomous keys and published literature (M. A. Price & Graham, 1997; R. D. Price et al., 2003). Mites were taxonomically identified to family as previously described (J. M. Brennan & Goff, 1977).

To evaluate the nutritional condition of each bobwhite, the amount of breast muscle, subcutaneous fat, and visceral fat stores were subjectively scored during necropsy on a 0-6 scale, with “0” correlating to extreme emaciation and “6” to obesity (Dunn, 2003). All organs were examined for gross lesions, and tissues containing any suspect lesions were collected and stored in 10% neutral buffered formalin for histological examination. In addition, a standard tissue set (i.e., samples 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) was collected from a randomly selected subset of 20% of quail from each WMA for standard histologic examination (histologic evaluation of head tissues is included in Chapter 4). Formalin-fixed tissues were routinely trimmed, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin using standard methods at the Athens Veterinary Diagnostic Laboratory, an AAVLD-accredited laboratory. Tissues from all birds were blindly evaluated microscopically by a board-certified veterinary pathologist.

Additional biological samples were collected for targeted pathogen screening. Tracheal swabs were collected for *Mycoplasma* spp. and *Trichomonas* spp. testing. The entire gastrointestinal tract was removed and examined for parasites (these results are reported in Chapter 4). The spleen was collected for *Haemoproteus* spp., *Leucocytozoon* spp., *Plasmodium* spp., *Pasteurella multocida*, and reticuloendotheliosis virus testing. Breast muscle tissue and a portion of the small intestine were collected for *Sarcocystis* spp. and *Eimeria* spp. testing, respectively. These samples were frozen to -20C prior to DNA extraction. For virus isolation on Vero cell monolayers, small portions of heart, kidney, and brain were pooled and homogenized in tubes containing virus isolation media (Allison et al., 2004). For birds from which blood was not previously collected from the jugular vein, clotted blood from the heart was collected postmortem using a pipette and stored frozen for subsequent *Toxoplasma gondii* serologic testing.

Molecular assays for pathogen detection

Quail were tested for select infectious agents and parasites by PCR test as described in Table 3.1. Genomic DNA was isolated from the spleen, intestine, muscle tissue, tracheal swabs or parasites using a commercial kit and following manufacturer's instructions (DNEasy Blood and Tissue kit, Qiagen, Germantown, MD). PCR amplicons were separated using electrophoresis with a GelRed stained 1.5% agarose gel (Biotium, Fremont, CA). Gels were visualized using UV light from an AlphaImager (ProteinSimple, San Jose, CA). Amplicons were extracted from the gel using a gel extraction kit (Qiagen) and submitted to Genewiz (South Plainfield, NJ) for bi-directional sequencing. Sequences were cleaned with Geneious (Aukland, New Zealand) and compared with sequences in GenBank. Precautions were taken to prevent and detect contamination including performance of DNA extraction, PCR reaction setup, and product analysis in distinct and separate areas of the laboratory. Negative (water) controls were included in each batch of DNA extractions as well as for each set of PCR reactions. An appropriate positive control for each pathogen was included in each PCR batch.

Table 3.1 PCR primers used for the detection of selected pathogens in bobwhite quail (*Colinus virginianus*) from western Oklahoma.

Pathogen (gene target)	Gene target and Amplicon size	Primers	Reference
Mycoplasma	16S rRNA, ~490 bp	GMF-1 ('5- ACA CCA TGG GAG CTG GTA AT-3′) GMR-1 ('5- CCT CAT CGA CTT TCA GAC CCA AGG CAT-3′)	McGuire et al. 2014

Pasteurella multocida	KMT1, ~590 bp	KMT1T7 (‘5- ATCCGCTATTTACCCAGTGG-3′) KMTT1SP6 (‘5- GCTGTAAACGAACTCGCCAC-3′)	Townsend et al. 2001
Blood parasites (all 3 genera: *Haemaproteus*, *Plasmodium*, and *Leucocytozoon*)	cyt-b primary PCR, 617 bp	HaemNF1 (5’-CATATATTAAGAGAAITATGGAG-3′) HaemNR3 (5’-ATAGAAAGATAAGAAATACCATTC-3′)	Hellgren, Waldenström, and Bensch 2004
Blood parasites (*Haemaproteus* and *Plasmodium*)	cyt-b secondary PCR, 479 bp	HaemNF (5’-CATATTAAGAGAATTATGGAG-3′) HaemNR2 (5’- AGAGGTGTAGCATATCTATCTAC-3′) or HaemF (5’- ATGGTGCTTTCGATATATGCATG-3′) HaemR2 (5’- GCATTATCTGGATGTGATAATGGT-3′)	Hellgren, Waldenström, and Bensch 2004
Blood parasites (*Leucocytozoon*)	cyt-b secondary PCR, 479 bp	HaemFL (5’-ATGGTGTTTTAGATACTTACATT-3′) HaemR2L (5’-CATTATCTGGATGAGATAATGGIGC-3′)	Hellgren, Waldenström, and Bensch 2004
Trichomonas	ITS1-5.8S-ITS2, 330-391bp	TRF-1 (‘5-TGCTTCAGTTCAGCGGGTCTTCC-3′) TRF-2 (‘5- CGGTAGGTGAACCTGCCGTTGG-3′)	Hayes, Anderson, and Walker 2003
Sarcocystis	18S rRNA, ~ 700 bp	18S9L (‘5- GGATAACCTGGTAATTCTATG-3′) 18S1H (5’-GGCAAATGCTTTCGCAGTAG-3′)	Li et al. 2002
Intestinal *Eimeria* spp.	18S rRNA, ~ 700 bp	18S9L (‘5- GGATAACCTGGTAATTCTATG) 18S1H (5’-GGCAAATGCTTTCGCAGTAG-3′)	Li et al. 2002
Physaloptera spp. (COI)	COI, 557 bp	PhyF (5'-GGGCAGGATTAGGAGGTTCTG-3') PhyR (5'-AAGCCCCAGCCAAAACTGGAA-3')	Kalyanasundaram et al. 2018
Reticuloendotheliosis virus	Envelope gene, ~ 291 bp	SNVUP (5'-CATACTGGAGCCAATGGTT-3′) SNVLO (5'-AATGTTGTACCGAAGTACT-3′)	Aly et al., 1993
West Nile virus	Nucleocapsid and premembrane genes, 376 bp	WN310F (5′-GTSAACAAAACAAACAGCRATGAA-3′) WN686R (5′-ACWGMTGAYTTYGTGCACCA-3′)	Allison et al. 2004

Serologic testing for select pathogens

Adenovirus. To screen for antibodies to adenoviruses by ELISA test, serum samples were routinely processed according to standard protocols at the Poultry Diagnostic and Research Center (PDRC) at the University of Georgia (Athens, GA).

Toxoplasma gondii. A modified agglutination test (MAT) was conducted on whole heart blood collected during necropsy to detect antibodies to *T. gondii* as previously described (Dubey & Desmonts, 1987).

Virus isolation and serologic testing for West Nile virus

Virus isolation. Isolation of flaviviruses from a sample of pooled heart, kidney, and brain tissues was conducted in confluent, 2-day old Vero cell culture monolayers as described (Allison et al. 2004). Any samples that displayed cytopathic effects within 10 days were further evaluated using VecTest antigen assay (Vectests, Medical Analysis Systems, Camarillo, CA) to test for West Nile virus antigen. If VecTest results indicated the presence of West Nile virus antigen, then a RT-PCR was conducted to confirm the presence of WNV RNA presence as described (Table 3.1).

Plaque reduction neutralization (serologic) test for antibodies to West Nile virus. Nobuto strips were eluted to a dilution of 1:10 per the manufacturer's instructions. Nobuto strip eluates were heat inactivated at 56°C in 5% CO_2 for 30 minutes, centrifuged at 12,000 x g for 4 minutes, and subsequently frozen at -20°C until testing. Nobuto strip eluates were tested for anti-WNV antibodies by plaque reduction neutralization test as previously described (Allison et al., 2004), with the following modification. Cultures were inactivated on day 5 post adsorption with 10% buffered formalin and stained with 0.25%-1% crystal violet for plaque visualization. Samples that neutralized <90% of WNV plaque forming units (PFU, $PRNT_{90}$) compared with control wells at a 1:20 dilution were considered negative for anti-WNV antibodies.

Eluates with ≥90% neutralization were co-titrated against both WNV and St. Louis encephalitis virus (SLEV) to determine the causative virus by at least a four-fold $PRNT_{90}$ titer. Titers were expressed as the reciprocal of the highest dilution at which ≥90% WNV plaque forming units (PFU, $PRNT_{90}$) were neutralized as compared with control well samples (i.e., viral media with virus).

Gross parasite assessment

The breast muscles of all quail were grossly examined for intramuscular parasites by checking the surface of the pectoral muscle, then carefully separating the two layers of muscle, i.e., the pectoralis and supracoracoideus muscles (Biewener, 2011; Tobalske, 2016). The two layers of pectoral muscles were "bread loafed" or serially sectioned to assess for intramuscular parasites. The muscles of the legs were similarly examined for intramuscular parasites. Any intramuscular parasites found were stored in 70% ethanol for later identification. When numerous intramuscular parasites were observed in the same tissue and anatomic location, some of the parasites were excised along with surrounding tissue and stored in 10% buffered formalin for histological examination. Intramuscular nematodes were morphologically identified as *Physaloptera* (Boggs et al., 1990) so to better characterize this parasite, genetic characterization was conducted as described (Table 3.1).

Toxicology Testing

A subsample of 21 whole bobwhite 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 that included: arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), lead (Pb), zinc (Zn) and organic chemicals. For analysis of heavy metals, 1 g of liver was digested with 3ml of nitric acid at 190C. After the digestion was completed, 2 mL of hydrochloric acid was added, and the sample was brought to 10 mL with 18Mohm water. The sample was then analyzed by ICP-OES (inductively coupled plasma optical emission spectrometry). To ensure data quality, a method blank, laboratory control spike, sample over-spike, and a CRM (certified reference material from the National Research Council of Canada) was digested and analyzed with each batch. For every ten samples, a drift check was also run to ensure instrument stability. The detection limits for each heavy metal were as follows; 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 (GC/MS) and liquid chromatography-mass spectrometry (LC/MS). These screens are designed to detect hundreds of diverse organic compounds from different chemical categories, including pesticides, environmental contaminants, drugs and natural products. For GC/MS screening, liver samples were homogenized in 5% ethanol in ethyl acetate, centrifuged, and a portion of the extract was then evaporated dry and reconstituted in 30% ethyl acetate in hexane. This solution was purified using a gel permeation chromatography system (J2 Scientific, Columbia, MO) equipped with S-X3 Bio-Beads (Bio-Rad, Hercules, CA). Extracts were then analyzed by GC-MS on an Agilent 6890-5975 system. The GC was fitted with a 30 m Agilent DB-5 column using a temperature program that started at 40C and ramped to 290C over the course of a 32 minute run. The mass spectrometer was operated in full scan electron ionization mode, scanning from m/z 45 to m/z 650. Automated software was used to detect peaks in the total ion chromatogram and search their spectra against the Wiley mass spectral library (11th Ed., John Wiley and Sons, Hoboken, NJ). Automated Mass Spectral Deconvolution and Identification System deconvolution, a 200nd library search software (NIST, Gaithersburg, MD) was also used to identify compounds present in the samples. Results generated by these two programs were reviewed by toxicology personnel to determine the presence of toxicants. The LC/MS was conducted as described (Filigenzi et al., 2011).

Results

Bobwhite collection and demographic data

From 2018-2020, 206 bobwhite quail from nine sites with a range of 1-56 quail per WMA and 42-91 quail per year were sampled (Figure 3.1). 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, adult bobwhite were in good nutritional condition based on muscle mass and fat stores, with average scores of 4.8 ± 0.96 for muscle mass and 3.4 ± 1.47 for fat stores. Breast muscles (pectoralis and supracoracoideus) 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 or muscle atrophy were rare or absent in these populations during the sampling period. The fat store scores of adult bobwhite collected from the earlier trapping seasons (*n* = 41, 3.37 ± 1.3) were similar to the fat store scores of adult bobwhite sampled from the later trapping season (*n* = 69, 3.41 ± 1.57). The weights of male and female adult bobwhite varied slightly year to year, although the overall average body weights of the two sexes were nearly identical (181.1g and 180.8g, respectively; Table 1).

No quail had significant gross lesions, such as those that may occur with wet or dry avian pox, avian influenza, gastroenteritis (e.g., clostridial), mycoplasmosis, or conjunctivitis. During necropsy, *Tetrameres* spp. were observed in the proventriculus, with prevalence rates of 0%, 2%, and 10% in 2018, 2019, and 2020, respectively. *Tetrameres* spp. intensities (i.e., 5 parasites per infected bobwhite) ranged from 1-15 with a mean intensity of 5. Both eyeworms and gastrointestinal parasites were observed during necropsy, and these results are reported in Chapter 4 of this thesis.

Table 3.2 Average weights of adult male and female bobwhite quail (*Colinus virginianus*) sampled in western Oklahoma.

	Average Body Weights			
Adult Bobwhite	**2018 (37,22)***	**2019 (23,13)***	**2020 (10,5)***	**Overall (69,39)***
Males	178.1g ± 15.5	185.9g ± 11.7	181.5g ± 7.6	181.1g ± 13.8
Females	179.5g ± 17.8	183.5g ± 11.1	180g ± 6.2	180.8g ± 14.6
Overall	178.6g ± 16.2	185g ± 11.4	180.7g ± 6.9	181g ± 14

*Sample sizes for males and females, respectively.

Overall, juvenile bobwhite were in good nutritional condition based on muscle mass and fat stores, with average nutrition scores of 3.6 ± 0.87 for muscle mass and 2.3 ± 0.98 for fat stores. The muscle mass score of juvenile quail was significantly lower than that of adults (t(204)=9.18, p<0.0001), but most juvenile bobwhite had healthy amounts of breast muscle. Almost all of the juveniles with less than the average muscle mass score were very young (i.e., <50 days of age). No juveniles exhibited severe malnutrition or muscle atrophy. The fat store scores of juvenile bobwhite were significantly lower compared to those of adults (t(191)=6.2,p<0.0001) with nearly 15% (n=14) of juvenile bobwhites having little to no visible fat stores.

Organic compound and heavy metal screening

No toxins were detected in the livers of 21 bobwhite that underwent general toxin screening at the CAHFS Laboratory (Table 3.3). Livers of the 11 bobwhite that were screened for heavy metals had no evidence of arsenic, lead, mercury, and cadmium but low levels of iron, manganese, zinc, copper, molybdenum were detected.

Table 3.3 Results of toxin and heavy metal screening of bobwhite quail (*Colinus virginianus*) from western Oklahoma.

Toxicants	Detected/Total Screened (%)	Average Detection (ppm)	Range of Detection (ppm)
Organic Compounds	0/21 (0%)	n/a	n/a
Lead	0/11 (0%)	n/a	n/a
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%)	n/a	n/a
Arsenic	0/11 (0%)	n/a	n/a

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
Cadium	0/11 (0%)	n/a	n/a

*Organic compounds - (chemicals originating from pesticides, environmental; n/a = not applicable contaminants, drugs and natural products)

Pathogen Screening

Viral Pathogens

Testing for WNV included both serology (antibodies) and virus isolation (infectious virus). No viruses were isolated from pooled tissues from the 206 bobwhite tested. In addition, a total of 38 bobwhite were screened for antibodies to WNV and all were negative. None of the 206 bobwhite tested by PCR for reticuloendotheliosis virus had viral DNA detected. Antibodies to group I serotype 1 avian adenovirus (quail bronchitis virus) were detected in 4 of 46 (8.6%) bobwhite (Table 3.4).

Table 3.4 Results of molecular and serologic testing for select pathogens in bobwhite quail (*Colinus virginianus*) from western Oklahoma in 2018-2020.

			No. infected bobwhite/No. Sampled (%)			
Pathogen	**Pathogen Type**	**Test Method***	**2018**	**2019**	**2020**	**Overall**
Avian adenovirus	Virus	Serology (ELISA)	n/a	3/15 (20%)	1/31 (3%)	4/46 (8.6%)
Reticuloendotheliosis virus (REV)	Virus	PCR	0/73 (0%)	0/91 (0%)	0/42 (0%)	0/206 (0%)

West Nile virus	Virus	Isolation/ Serology (PRNT)	0/73 (0%)	0/91 (0%)	0/42 (0%)	0/206 (0%)
Mycoplasma spp.	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	0/73 (0%)	0/91 (0%)	0/42 (0%)	0/206 (0%)
Leucocytozoon spp.	Protozoa	PCR	0/73 (0%)	0/91 (0%)	0/42 (0%)	0/206 (0%)
Plasmodium spp.	Protozoa	PCR	0/73 (0%)	0/91 (2%)	0/42 (0%)	0/206 (0%)
Toxoplasma gondii	Protozoa	Serology (MAT)	n/a	1/91 (1%)	2/42 (5%)	3/133 (2.3%)
Trichomonas spp.	Protozoa	PCR	n/a	0/91 (0%)	0/42 (0%)	0/133 (0%)

*PCR=polymerase chain reaction, MAT=modified agglutination test, PRNT=plaque reduction neutralization test, ELISA= enzyme-linked immunosorbent assay.

Bacterial Pathogens

None of the 133 bobwhite tracheal swabs tested for *Mycoplasma* spp. and *Pasteurella multocida* in 2019 and 2020 had evidence of the respective bacterial DNA.

Protozoan Pathogens

None of the 206 spleen samples had evidence of *Haemoproteus*, *Leucocytozoon*, or *Plasmodium* spp. DNA. Similarly, tissues from none of the 133-bobwhite tested had evidence of *Trichomonas* spp. or *Eimeria* spp. genetic material. However, three of the 133 (2.3%) bobwhite had antibodies to *T. gondii*.

Intramuscular Parasites

We observed 2 intramuscular parasites, including a *Physaloptera* sp. and a *Sarcocystis* sp. The *Physaloptera* sp. was observed in the breast and leg muscles of 7-12% of bobwhite from 2018-2020. *Physaloptera* sp. intensities ranged from 1-36 with a mean intensity of 5.2 and decreased annually from 7.7 in 2018 to 0 in 2020. Single *Sarcocystis* sarcocysts were observed grossly during necropsy in two (1%) bobwhite. PCR testing of all 206 muscle samples tested were negative for *Sarcocystis* DNA. PCR was not conducted on the two sarcocysts observed grossly as they were preserved in formalin for histologic examination.

Genetic characterization was carried out on 20 individual *Physaloptera* larvae. Fourteen unique sequences (550bp) were obtained. 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). Intraspecific variation was low (98.5-99.8% identical). The sequences were most similar to a *Physaloptera* sp. previously detected in a quail from Mitchell County, Texas (LC381943) (97.7-98.9% identical) (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 (MW517846) (97% identical) followed by *P. retusa* (86% identical). Our sequences also were a close match (i.e., shorter overlap of sequences) to two *Physaloptera* sp. sequences from grasshoppers in Mitchell County, Texas (98-99% identical for 424 bp, LC596961 and LC596962).

Tetrameres spp. were observed within in the lumens of proventricular glands in 3% of the examined bobwhite, with a mean intensity of 5 ± 5.2 and a range of intensity of 1-15. When examined histologically, the embedded *Tetrameres* spp. appeared to cause no inflammation or other tissue changes in the surrounding tissues.

Table 3.5 Intramuscular and intraproventricular parasites detected in bobwhite from western Oklahoma in 2018-2020.

Parasite Species	Location of Infestation	No. infected bobwhite/No. Sampled (%) 2018	2019	2020	Overall	Range of Intensity	Overall Mean Intensity	Total Recovered
Sarcocystis spp.	Breast Muscle	0/73 (0%)	2/91 (2%)	0/42 (0%)	2/206 (1%)	1	1	2
Physaloptera sp.	Breast Muscle Leg Muscle	9/73 (12%)	12/91 (13%)	3/42 (7%)	24/206 (12%)	1-36	5.2 ± 7.3	114
Tetrameres spp.	Proventriculus	0/73 (0%)	2/91 (2%)	4/42 (10%)	6/206 (3%)	1-15	5 ± 5.2	29

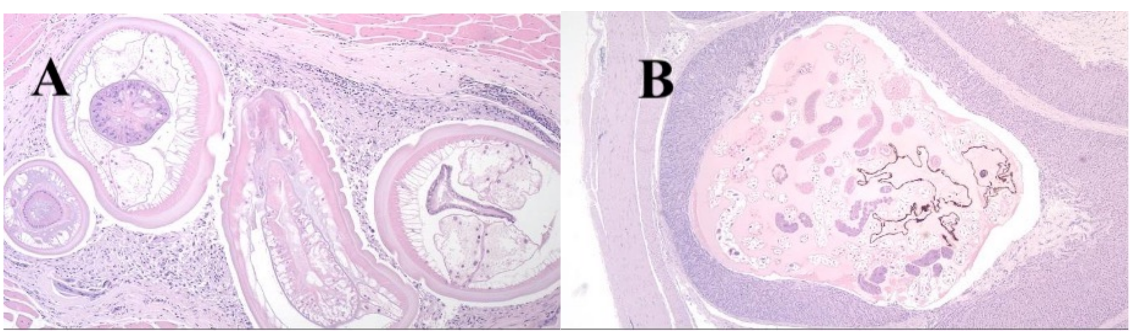

Figure 3.2. Photomicrographs of *Physaloptera* sp. and *Tetrameres* sp. A) *Physaloptera* sp. embedded in the skeletal muscle of the breast, with mild surrounding inflammation and an outer fibrous capsule, indicating chronicity. B) *Tetrameres* sp. expanding the glandular lumen of the proventriculus, with no associated inflammation.

External parasites

The prevalence of ectoparasites increased over time (i.e., by year), with overall prevalences of 30% (n = 73), 85% (n = 91), and 98% (n = 42) in 2018, 2019 and 2020, respectively. A total of three species of chewing lice (i.e., *Goniodes ortygis, Menacanthus* spp., *Oxylipeurus clavatus*) were observed on the skin of bobwhite. The most common chewing louse species observed was *G. ortygis* (39%) (Table 3.6). The other two chewing lice species were found at lower prevalences. A chigger (Trombiculidae) was the only mite observed on quail (6%).

Table 3.6 Ectoparasites recovered from bobwhite in western Oklahoma in 2018-2020.

		No. infected bobwhite/No. Sampled (%)						
Parasite Species	**Parasite type**	**2018**	**2019**	**2020**	**Overall**	**Range**	**Overall MI**	**Total Recovered**
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 sp.	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

Three species of tick (*Amblyomma americanum*, *A. maculatum*, and *Haemaphysalis leporispalustris*) were detected on bobwhite (Table 3.6). The rabbit tick, *H. leporispalustris,* was the most prevalent tick species observed with an overall prevalence of 30% and a total of 292 ticks recovered (266 larvae and 26 nymphs). The Gulf coast tick, *A. maculatum,* was the second most prevalent tick species observed with an overall prevalence of 15% and 94 ticks recovered (87 larvae and 7 nymphs). Finally, the lone star tick, *A. americanum,* was rarely observed with only four larvae detected on 2 (1.5%) quail.

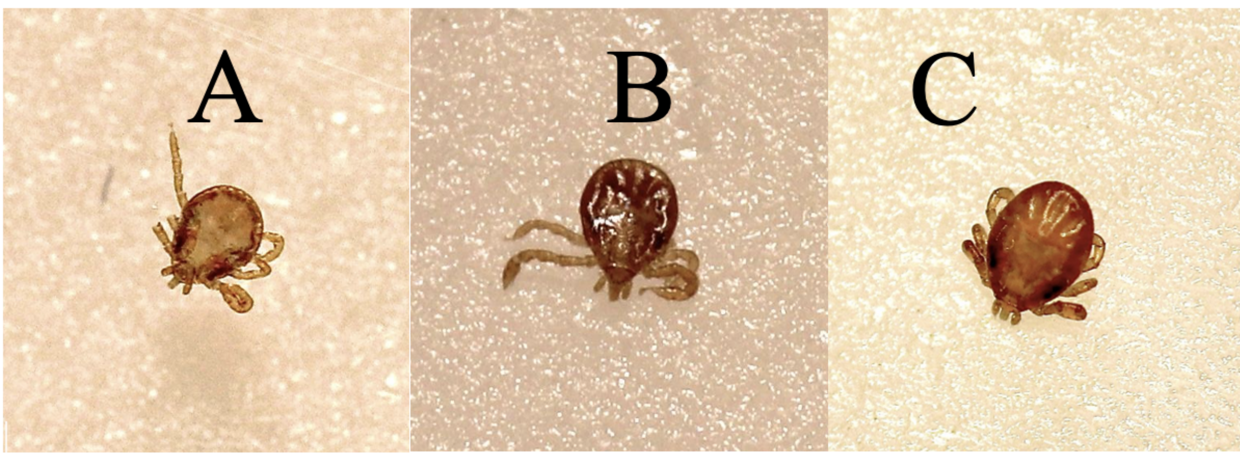

Figure 3.3. The three species of ticks found on bobwhite in Oklahoma. A) *Amblyomma americanum* larval tick. B) *Amblyomma maculatum* larval tick. C) *Haemaphysalis leporispalustris* larval tick.

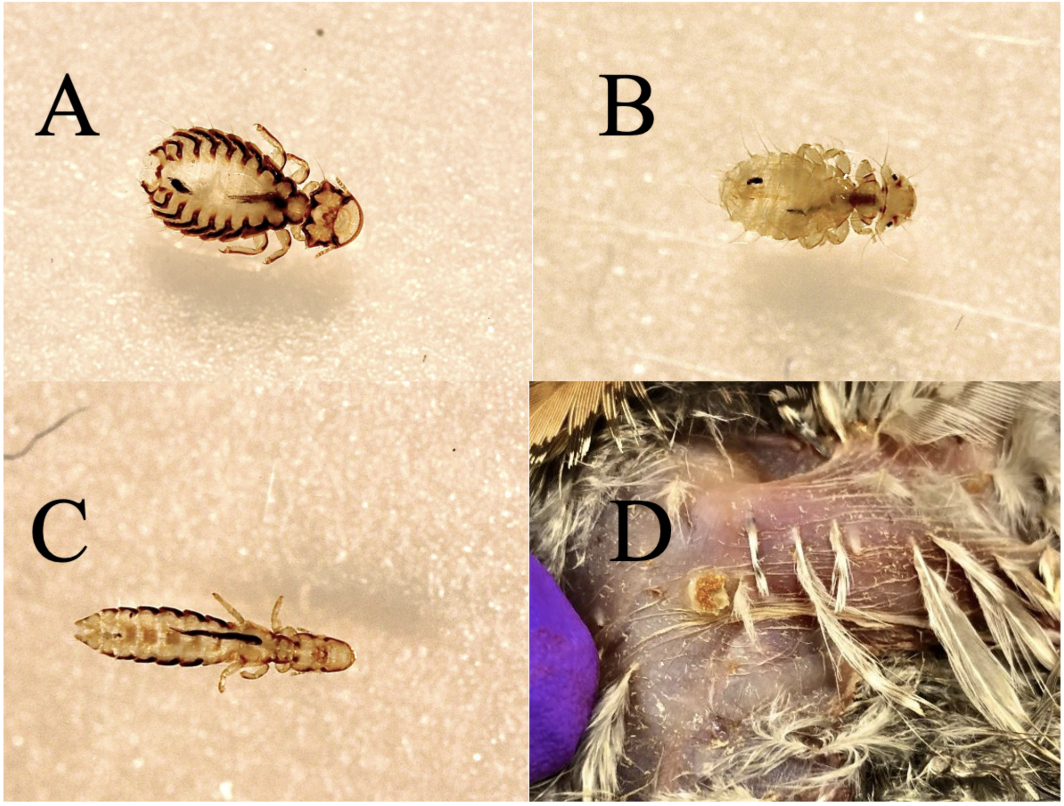

Figure 3.4. The three species of chewing lice and the single species of mite observed on bobwhite from Oklahoma. A) Chewing louse; *Gonoides ortygis*. B) Chewing louse; *Menacanthus* spp.; C) Chewing louse; *Oxylipeurus clavatus*; D) Mites; *Trombiculidae* (chiggers) are distinguished by a protruding orange mass on the skin of the bobwhite, which can be observed in the center-left of photo D.

Discussion

Oklahoma is widely regarded as one of the last strongholds for the Northern bobwhite quail, due to having sufficient quail numbers to attract hunters from other states. While bobwhite population numbers have been historically higher and/or more stable than surrounding states, the population of bobwhite in Oklahoma is currently experiencing a decline (Church et al., 1993). While bobwhite populations typically undergo a ‘Boom and Bust’ cycle, the booms have been decreasing in terms of quail numbers (Judkins, 2020b). To better understand the diversity and prevalence of selected pathogens in bobwhite quail in western Oklahoma, we evaluated the health of quail using various modalities including gross necropsy, histopathologic evaluation, and pathogen/parasite testing. These data show that the evaluated quail appeared to be in good general health, and that some pathogens and parasites of concern were detected (i.e., *Toxoplasma gondii* and adenovirus).

The average body weights of adult bobwhite remained relatively stable for the three years of the study. This stability suggests that sufficient food and other resources were consistently available despite extreme weather that occurred in 2019 (i.e., near record rainfall amounts) and 2020 (i.e., near drought conditions) in western Oklahoma. In addition, the average weights of both male and female bobwhite were similar to bobwhite sampled during a previous study also conducted in western Oklahoma (Andersson & Eric, 2021). Overall, adult and juvenile bobwhite had good muscle mass and fat store scores. On average, juvenile bobwhite had significantly less 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 bobwhite generally have a broader available prey base due to 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 likely 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).

Heavy metals such as copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and molybdenum (Mo) were detected in bobwhite tissue (liver), although lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), and organ chemicals were not detected. Only molybdenum had a level that was considered elevated, although this level was not considered remarkable or a health concern. However, continued surveillance is warranted because these compounds can impact quail health and we tested a subset of quail. Lead, mercury, and two organochlorine pesticides were previously detected in bobwhite from the Rolling Plains ecoregion of Texas and Oklahoma (Baxter et al., 2015).

Laboratory testing for numerous pathogens and parasites yielded negative results, further supporting the good general health status of sampled bobwhite in western Oklahoma. The lack of blood parasites (*Haemoproteus*, *Leucocytozoon*, and *Plasmodium* spp.) generally is consistent with previous studies of bobwhite in Texas and Oklahoma (Kocan et al., 1979; Peterson, 2007; Xiang et al., 2017). These parasites were also absent in bobwhite in studies in Florida and Iowa, but *Haemoproteus* and *Plasmodium* have been detected in bobwhite in Maryland and Georgia (Crook et al., 2009; F. E. Kellogg & Doster, 1972; Peterson, 2007). The lack of detection of *Haemoproteus* spp. in this study was noteworthy; one species (i.e., *Haemoproteus lophortyx*) can cause serious illness and death in captive bobwhite and masked quail (*Colinus virginianus ridgwayi*) and it has been detected in wild Gambel's (*Callipepla gambelii*) and scaled quail (*Callipepla squamata*) in Arizona, California, Colorado, Nevada, and New Mexico (Cardona et al., 2002; Peterson, 2007). The lack of detection of these vector-borne blood parasites could be related to an absence of the parasites and/or vectors in western Oklahoma. However, future surveillance is warranted as environmental, or climate change could alter the geographic ranges and habitat preferences of these parasites and/or vectors.

Similarly, there was no evidence of either active or previous infection with WNV, a mosquito-borne virus. A previous study in the Rolling Plains ecoregion of Texas found that a low percentage (4.8%) of wild-caught bobwhite had antibodies to WNV, which generally indicate survival from infection (Urban et al., 2013). Transmission of WNV can occur via a variety of mosquito species that vary across the landscape and in their host feeding preferences, so annual variations in environmental factors, including weather patterns, can greatly impact transmission (Komar et al., 2003). Adult bobwhite experimentally infected with WNV developed subclinical infection with low viremia titers and readily seroconverted, suggesting that bobwhites have low susceptibility to WNV-associated disease and are unlikely to play a role in mosquito-bird transmission cycles (Kunkel et al. in press). However, susceptibility of bobwhite chicks to WNV infection and disease has not been evaluated, and some age variation in manifestation of WNV infection has been observed in some bird species (Komar et al., 2003; Pérez-Ramírez et al., 2014; Urban et al., 2013). The lack of detection of *Trichomonas* spp. from the oral cavity was similar to a previous study in Texas and Oklahoma, although bobwhite are experimentally susceptible to infection (but not disease) with this protozoan (Andrea Bruno et al., 2015; Levine et al., 1941).

Antibodies to *T. gondii*, causative agent of toxoplasmosis, were detected at a low prevalence in quail screened. Although PCR testing was not conducted, numerous studies have shown that animals with antibodies to *T. gondii* (i.e., evidence of past infection) often have viable parasites in tissues (Dubey et al., 1994; El Behairy et al., 2013; Herrero et al., 2016). This parasite presents a potential health concern in bobwhite, as they are highly susceptible to experimental infection and can develop acute and chronic forms of potentially fatal clinical disease. In one study, 25% of bobwhites inoculated with a high dose of *T. gondii* died within a week (Dubey, 2002; Dubey et al., 1993; Hill & Dubey, 2002). Other quail species, such as California quail (*Callipepla california*) and Japanese quail (*Coturnix japonica*), are also highly susceptible to *T. gondii* infection and can develop severe, sometimes fatal disease (Casagrande et al., 2015; Dubey et al., 1994). Despite the possibility of severe toxoplasmosis in bobwhite, there are few surveys for *T. gondii* in wild populations of bobwhite, although toxoplasmosis was diagnosed in four wild quail from Georgia evaluated at SCWDS (Davidson et al., 1982). The low prevalence of *T. gondii* detected in our study could potentially reflect a high mortality rate associated with infections as shown in experimental trials; alternately, it may reflect a lack of pathogen circulation and thus transmission in the region.

Antibodies to avian adenovirus, the causative agent of quail bronchitis, were detected in nearly 9% of quail in Oklahoma. Quail bronchitis can develop in bobwhite of all age groups and while adult bobwhite can survive infection, younger bobwhite often develop more severe disease, and chicks <3 weeks of age can experience high mortality rates (e.g., 50%-100%) (DuBose, 1967; Jack et al., 1994; Jack & Reed, 1990). Although there are several reports of avian adenovirus infection in captive bobwhite, data on free-ranging quail are limited. In a study in Florida, antibodies to avian adenovirus were detected in 23% of adult bobwhite, which is much higher than the ~9% prevalence we observed (King et al., 1981). Turkeys and chickens often are sub-clinically infected, which can facilitate the maintenance and spread of the virus in the environment (DuBose, 1967; Jack et al., 1994; Jack & Reed, 1990). The evidence of avian adenovirus infection in bobwhite in Oklahoma is a potential health concern, especially for chicks. Fatal infections in the latter could easily be missed as dead chicks are rarely found and submitted for diagnostic evaluation. However, this potential threat bears further investigation.

All of the external parasites we detected are considered common in bobwhite in Oklahoma and other regions. Although we noted an overall increase in prevalence throughout the study period, intensities of the chewing lice, mites, and ticks remained low. None of these ectoparasites are considered to present a health risk for bobwhites; however, *A. americanum* and

A. maculatum are important vectors for pathogens of significance to people and animals (Lockwood et al., 2018; Paddock & 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 (*H. longicornis*), as immature stages can look similar (Beard et al., 2018; Egizi et al., 2020; Thompson et al., 2020; White et al., 2020). The Asian longhorned tick was recently introduced the eastern United States, but has been reported as far west as northwestern Arkansas and Missouri, monitoring its spread has important public and veterinary health implications (USDA-APHIS, 2021).

Tetrameres spp. were observed in 3% of examined bobwhite, similar to studies in Texas (1%-26%) (Herzog et al., 2021; Olsen et al., 2016; Shea et al., 2020; Villarreal et al., 2016). All *Tetrameres* spp. in the present study were within the lumen of the proventricular glands. Histologically, these glands were dilated with no associated tissue damage. However, associated lesions (e.g., ulceration, severe inflammation) have been reported in the proventriculus when the parasites occur in high intensities (i.e., well above our observed maximum intensity of 15) (F. E. Kellogg & Doster, 1972). The single immature acanthocephalan could not be identified, but acanthocephalans are generally believed to uncommonly infect quail and thus, are not considered to be a health risk.

Physaloptera larvae have been reported in the breast muscle of bobwhite as far back as the 1930's and the potential for associated breast muscle damage has caused concern. We detected *Physaloptera* larvae in muscle tissue in 11% of quail, which is similar to studies in Oklahoma (8%) and Texas (23%) (Boggs et al., 1990; Herzog et al., 2021). We noted a decrease in *Physaloptera* sp. mean intensity from 7.7 to 1 during our study that may be related to densities of intermediate host prey. Although we did not see gross lesions associated with *Physaloptera*, which were limited to the breast and leg muscle in our study, Boggs et al. (1990) observed necrosis and granulomatous inflammation associated with larval migration through the tissue. Similarly, we observed minimal microscopic inflammation in bobwhite in Oklahoma, while a previous study in Oklahoma noted necrosis and granulomatous reactions in tissue associated with the parasite's presence (Boggs et al., 1990) This parasite likely represents an under-recognized cause of mortality when infection intensities are high; for example, SCWDS evaluated hunter-harvested bobwhite from Kansas with high numbers of nematodes in the breast muscle. Interestingly, three pheasants from the same area had myositis associated with *Physaloptera* in breast muscle.

The bobwhite serves as a paratenic host for the aforementioned *Physaloptera* sp. and, to date, no definitive host has been identified. A study in Texas genetically characterized *Physaloptera* detected in bobwhite, and although there was no match with existing sequences in GenBank, the sequences were similar to those detected in grasshopper intermediate hosts (Kalyanasundaram et al., 2018). Our sequence data showed that the Oklahoma *Physaloptera* parasites were the same species as those from Texas. We also found a high diversity of unique haplotypes, although intraspecific variation was low. However, these new genetic data fail to elucidate potential definitive host(s) for this parasite. Interestingly, the closest match currently available in Genbank is from a feral cat in India, but the quail *Physaloptera* sp. sequences were not a close match for any North American sequences, including unpublished sequences from *Physaloptera* spp. from raccoons, opossums, coyotes, dogs, and cats in the United States (Yabsley, unpublished data). Molecular characterization of *Physaloptera* from additional mammalian, avian, and reptile hosts in the region is needed to identify the definitive host for the quail *Physaloptera* sp. Additionally, studies on factors related to *Physaloptera* transmission are needed as higher intensities likely can cause morbidity that may increase predation risk.

Research on the health status of a populations of target wildlife species, bobwhite in this case, can provide critical data for selecting the best management strategies. Knowledge on the diversity and prevalence of pathogens and parasites is a base for understanding the risk of disease to bobwhites. We found that free-ranging bobwhite 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 of the potential pathogens (e.g., adenovirus) and parasites (e.g., *T. gondii*) detected have reportedly been associated with health impacts in bobwhite in other regions (A. Bruno et al., 2019; Herzog et al., 2021; Villarreal et al., 2016); thus, further investigations on potential population health risks are needed to assist in future disease surveillance and management strategies in bobwhite.

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CHAPTER 4

GASTROINTESTINAL AND OCULAR PARASITES OF NORTHERN BOBWHITE QUAIL (*COLINUS VIRGINIANUS*) IN WESTERN OKLAHOMA[1]

[1]Wyckoff, S.T., Judkin,Tell., Nemeth, N, Ruder, M.G., Martin, J., Yabsley, M.J. 2021. To be submitted to Veterinary Parasitology: Regional Studies and Reports

Abstract

The Northern Bobwhite Quail (*Colinus virginianus*) is a popular upland game bird in Oklahoma that is suffering from severe and ongoing population decline. In this study, we investigated the potential health impacts of gastrointestinal and periorbital parasites in bobwhite quail in western Oklahoma. From 2018-2020 a total of 206 bobwhite were sampled and overall, a low prevalence and diversity of parasites were detected. However, at least one gastrointestinal or ocular parasite species was detected in 212 bobwhite. This included three gastrointestinal parasite species, including *Aulonocephalus pennula* (54% prevalence, mean intensity 71.6 ± 99.8), *Raillietina* spp. (7%, 4.2 ± 1.9), and a single unidentified immature acanthocephalan (0.5%). High burdens of *A. pennula* and *Raillietina* infections were significantly associated with reduced fat stores in their bobwhite host. Eyeworms (*Oxysprirua petrowi*) were observed in 12.6% (26/206) of bobwhite sampled and intensities were low (range 1-10, mean 3.9 ± 2.9). No significant histologic lesions were associated with *O. petrowi* worms in various ocular structures. Overall, we found that the prevalence and intensity of parasites in bobwhite quail in Oklahoma were lower than in previous studies in similar physiographic regions of Texas. However, continued studies on the impacts of these parasites on quail health are needed as environmental and climate changes could alter the ecology and significance of these parasites.

INDEX WORDS: Northern Bobwhite Quail, population decline, parasites, *Colinus virginianus, Oxyspirura petrowi*, *Aulonocephalus pennula*, *Raillietina*

Introduction

The Northern Bobwhite Quail (bobwhite, *Colinus virginianus*) is one of the most popular game species in Oklahoma (Kellogg & Calpin, 1971). Range wide, bobwhite populations have been declining with a complete disappearance in some northern areas of their range (Hernández et al., 2013). Annually, the bobwhite population has undergone a ~4% decline per year, resulting in an 85% decline in the population since the mid 1960's (Sauer et al., 2017). With the ongoing decline across much of their range, the need for research on risks to bobwhite health are critical.

Oklahoma has one of the highest remaining densities of bobwhite, and these populations are also experiencing a 31-year statewide decline (Janus, 2018; Judkins, 2020a, 2020b). Annual statewide roadside surveys illustrate a 'Boom and Bust' cycle of the quail populations in Oklahoma, but there is an overall decrease in quail numbers (Judkins 2020b).

Numerous factors have been associated with bobwhite declines (e.g., habitat loss and change, climate and weather, etc.), but pathogens are always a concern for wildlife populations, especially those under stress from other factors. Numerous surveys of parasites infecting quail throughout their range exist, but more recently, several studies have focused on the possible impacts of *Aulonocephalus pennula* (caecal worm) and *Oxyspirura petrowi* (quail eyeworm) on quail in Texas (A. Bruno et al., 2019; Brym et al., 2018b; Dunham, Reed, et al., 2016; Dunham, Henry, et al., 2017; Dunham, Peper, et al., 2017; Shea et al., 2020; Villarreal et al., 2016). These studies suggest that infections with *A. pennula* and *O. petrowi* result in an increase in morbidity and mortality of infected bobwhite (Brym et al., 2018b; Dunham et al., 2014; Dunham, Reed, et al., 2016; Dunham, Peper, et al., 2017; Henry et al., 2017; Kalyanasundaram et al., 2019).

The purpose of this study was to establish a baseline for parasite prevalence and diversity in bobwhite from western Oklahoma. We focused on gastrointestinal parasites and eyeworms given data on their importance during previous research in similar ecoregions in Texas.

Methods

Animal capture, handling, and sampling

From 2018-2020, bobwhite quail were sampled from nine Wildlife Management Areas in the western region of Oklahoma (Figure 3.1). Each year, 24” x 24” x 8” funnel wire traps (Smith et al., 1981) were deployed during two 2-week sessions in early August and mid-October, for a total of 6 trapping sessions. Traps were pre-baited with a mixture of corn and seed two weeks to one month before the trapping period to increase capture success.

Bobwhite were euthanized by CO_2 inhalation followed by cervical dislocation and carcasses were immediately frozen at -20 C until necropsy. All animal trapping and handling techniques were reviewed and approved by the University of Georgia Institutional Animal Care and Use Committee (A2018 04-001 and A2020 11-010).

Parasite screening

The periorbital cavities of the bobwhite were grossly examined for the presence of *Oxyspirura petrowi* by using a small pair of forceps and any nematodes found were stored in 70% ethanol. Heads of bobwhite found to contain *O. petrowi* were preserved in Davidson’s Fixative (Poly Scientific R&D Corp, Bay Shore, NY) for subsequent histologic examination. Heads of *O. petrowi* infected bobwhite were decalcified before being routinely trimmed for histopathology with a focus on attaining multiple bilateral cross sections of the eye, periocular tissues, and orbit. Sections were then routinely embedded in paraffin, sectioned at 4 µm thickness, and stained with hematoxylin and eosin at the Athens Veterinary Diagnostic Laboratory, an American Association of Veterinary Laboratory Diagnosticians-accredited laboratory. Tissues of *O. petrowi* infected bobwhite were blindly evaluated microscopically by a board-certified veterinary pathologist. Results from evaluation of additional tissues from the same bobwhite are presented in Chapter 3 of this thesis.

The gastrointestinal tract from each of the necropsied quail was removed and divided into sections that included the small intestine, cecum, and large intestine. Each region was linearized, cut open longitudinally, and intraluminal contents were flushed onto a petri dish with saline solution. This material was carefully examined for parasites under a dissecting microscope, and any parasites found were preserved in 70% ethanol. Nematodes were identified to species and cestodes were identified to genus using dichotomous keys for morphology (Commons et al., 2019; Dunham, Henry, et al., 2017).

Results

Gastrointestinal Helminths

The most common gastrointestinal helminth detected was the caecal worm *Aulonocephalus pennula* (Table 4.1) with a range of intensity from 1-538 worms and an overall mean intensity of 71.6 ± 99.8. There was a significant annual increase in the mean intensity for *A. pennula*, with a mean intensity of 50.4 in 2018 and 103.5 in 2020 (Figure 4). Adult bobwhite observed to be infested with more than 200 *A. pennula* had significantly lower visible fat scores ($t(11)=-3.29$, $p=0.003$) than adult bobwhite with lower intensities. However, only 12.3% (n=10) of adult bobwhite had intensities of over 200 worms.

Tapeworms, all identified as *Raillietina* spp., were the second most common gastrointestinal parasite observed, with annual prevalences of 0-13%. The range of intensity for *Raillietina* spp. infections was 1-7 and an overall mean intensity of 4.2. The majority of bobwhite infected with *Raillietina* spp. was juvenile (92.9%, n=13). The juvenile bobwhite with

Raillietina spp. infections had significantly lower fat store scores (t(16)=-2.58, p=0.01) than juvenile bobwhite without *Raillietina* spp. infections.

The only other gastrointestinal parasite observed was a single immature acanthocephalan in a single bobwhite from 2019.

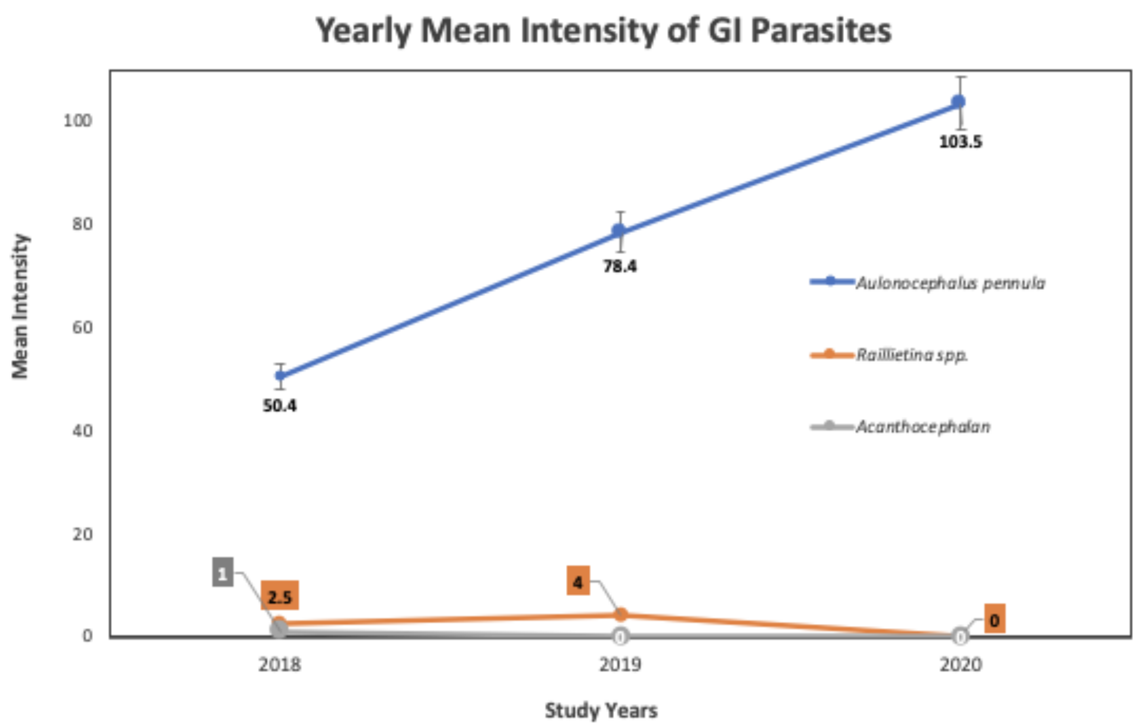

Figure 4.1 Annual mean intensities of gastrointestinal parasites in bobwhite in western Oklahoma from 2018-2020.

Ocular Parasites

We recovered *Oxyspirura petrowi* (quail eyeworm) from the eye tissues of 15%, 14%, and 5% of bobwhite collected from 2018, 2019, and 2020, respectively (Table 4.1). The overall prevalence of *O. petrowi* infections was 12.6%. The range of intensity for *O. petrowi* was 1-10 with an overall mean intensity of 3.9 eyeworms per infested bobwhite. The *O. petrowi* intensities varied by year with the highest intensity (5) in 2018 (intensities for both 2019 and 2020 were 3).

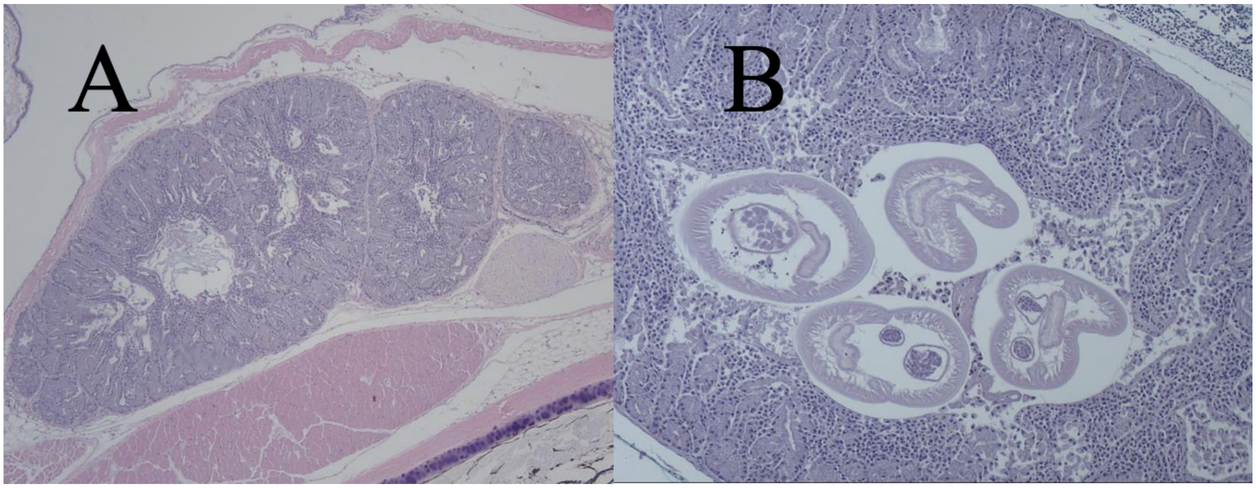

Figure 4.2 Accessory lacrimal (tear) glands (i.e., Harderian glands) with and without *Oxyspirura petrowi*. A) Harderian gland of a bobwhite without *O. petrowi* infection, with mild observable dilation. B) Harderian gland with intraluminal *O. petrowi*, with appropriate dilation to accommodate the presence of the parasite, with no associated evidence of inflammation or other cell reaction or damage.

Table 4.1 Gastrointestinal and ocular parasites recovered from bobwhite.

Parasite Species	Location of Infestation	No. infected bobwhite/No. Sampled (%) 2018	2019	2020	Overall	Range of Intensity	Overall Mean Intensity	Total Recovered
Aulonocephalus pennula	Gastrointestinal tract	47/73 (64%)	45/91 (50%)	20/42 (48%)	112/206 (54%)	1-538	71.6 ± 99.8	7944
Raillietina spp.	Gastrointestinal tract	2/73 (3%)	12/91 (13%)	0/42 (0%)	14/206 (6.7%)	1-7	4.2 ± 1.9	53
Acanthocephalan	Gastrointestinal tract	1/73 (1%)	0/91 (0%)	0/42 (0%)	1/206 (<1%)	1	1	1
Oxyspirura petrowi	Periorbital cavities	11/73 (15%)	13/91 (14%)	2/42 (5%)	26/206 (12.6%)	1-10	3.9 ± 2.9	103

Discussion

Oklahoma is widely regarded as one of the last strongholds for the Northern Bobwhite Quail, in terms of having sufficient quail numbers to lure in hunters from other states. While bobwhite population numbers have been historically higher and/or stable than surrounding states, the population of bobwhite in Oklahoma is currently experiencing a decline (Church et al., 1993). While bobwhite populations undergo a ‘Boom and Bust’ cycle, the booms have been decreasing in size (i.e., quail abundance) (Judkins, 2020b). To better understand the prevalence, intensities, and impacts of parasitic infections in bobwhite quail in western Oklahoma, we conducted parasite identifications and quantifications in targeted anatomic locations (i.e., gastrointestinal tract and periocular) and analyzed how these infections affected individual bobwhite health. These data show that the evaluated quail were in generally good health, and that some parasites of concern were detected in high burdens but in a limited number of cases. Additionally, the results of this study provide baseline health data for bobwhite quail in this region.

Caecal worms, *A. pennula,* were detected in each year of the study and were the most prevalent gastrointestinal parasite detected. Although over 50% of quail were infected in our study, prevalence was much lower than in previous studies in Texas in which prevalences were at or near 100% (Brym et al., 2018b; Dunham, Henry, et al., 2017; Herzog et al., 2021). In our study, the range of intensity was 1-538 and the average mean intensity of *A. pennula* in adult bobwhite increased each year of our study (maximum of 104 in 2020). The mean intensity of caecal infections in 2020 was double that of 2018. This drastic increase could be due to an increase of availability of intermediate hosts and an increase of monthly rainfall that peaks the transmission of heteroxenous nematodes such as *A. pennula* (Blanchard et al., 2019). Annual variation was also observed in a study in Texas that reported mean intensities of 111 and 372 during 2016 and 2017, respectively (Henry et al., 2017). Another study in Texas reported a much higher average intensity of 599 worms (Brym et al., 2018a). These mean intensities reported in bobwhite in Texas are much higher than what we observed in our study. However, we observed that adult bobwhite that had *A. pennula* intensities exceeding 200 worms had significantly lower fat scores than adult bobwhite with lower worm intensities. In those areas of Texas where the average worm intensity is well over 300 worms and nearly every bobwhite sampled is infected (Brym et al., 2018a; Henry et al., 2017), this parasite could be a health concern. Quail that have lower fat stores are less likely to survive through periods of reduced food availability as well as potential nutritional deficiencies due to severe *A. pennula* infections; the latter has been described in bobwhite inhabiting the Rolling Plains ecoregion of Texas (Brym et al., 2018b). Although ten quail in our study had severe (>200) *A. pennula* infections, none exhibited gross signs of emaciation. In our study, high caecal worm intensities were relatively rare but increased over time (annually) throughout the study, so continued efforts to understand the possible impacts of this parasite on quail populations are needed.

Raillietina spp. (tapeworms) were the second most detected gastrointestinal parasite in Oklahoma bobwhite. The detected prevalence of 7% was similar to those documented in bobwhite in Texas (9%-13%), although our mean intensity of 4 was higher than those in previous studies for which the mean intensities were from 1–1.9 (Herzog et al., 2021; Olsen & Fedynich, 2016; Shea et al., 2020; Villarreal et al., 2016). A significant age association was noted for *Raillietina*-infected quail, with predominantly juveniles infected. Also, infected juvenile bobwhite had significantly lower fat stores than uninfected juveniles; therefore, the potential impacts of *Raillietina* spp. on juvenile bobwhite should be investigated.

A single immature acanthocephalan was observed in one Oklahoma bobwhite but could not be identified. Acanthocephalans are generally believed to be uncommon in quail and thus, are not considered to be a health risk. The lack of detections in our study supports this notion.

Eyeworms are considered by many to pose a threat to bobwhite health. Although we detected *O. petrowi* in bobwhite in all three years of the study, the prevalence (5-15%) was lower compared to more recent studies in Texas (86%-100%) (Brym et al., 2018b; Henry et al., 2017; Herzog et al., 2021; Shea et al., 2020). Similarly, the mean worm intensity in bobwhite in our study (i.e., 4) and range of intensity (i.e., 1-10) were much lower than those reported in Texas (mean 6-44, range 1-107) (Dunham, Peper, et al., 2017). Similar to the Texas studies, we detected most *O. petrowi* infections in adult bobwhite (Andrea Bruno et al., 2015; Brym et al., 2018a; Dunham, Bruno, et al., 2016). This age relationship is likely due to adult bobwhite being better able to catch the intermediate host of *O. petrowi* (crickets and grasshoppers) and that it takes nearly 50 days for a *O. petrowi* to mature in a bobwhite and become observable in the periorbital space (Kalyanasundaram et al., 2019; Kistler et al., 2016).

Given the anatomic location of eyeworms in the host, any associated tissue damage and even the presence of the worms themselves has the potential to impact vision and thus, adversely affect foraging ability and predator avoidance (Jackson, 1969). Studies on eyeworms in quail from Texas reported that this parasite can cause inflammation and hemorrhage in periocular tissues (Andrea Bruno et al., 2015; Dunham et al., 2014; Dunham, Bruno, et al., 2016; Dunham, Reed, et al., 2016; Henry et al., 2017). In our study, *O. petrowi* were located in the lumen of the tear-producing gland (i.e., the Harderian gland) and/or between the eyelids (i.e., between the dorsal and ventral eyelids, and between the ventral eyelid and nictitating membrane). No gross or microscopic tissue damage was evident in the Harderian glands and eyelids of bobwhite that were infected with *O. petrowi*. For comparison, we also evaluated ocular and periocular tissues of uninfected quail as well as those of known infected quail with no worms detected (i.e., unilateral infections). Although Harderian glands with intraluminal worms were dilated, this is expected due to the presence of the worm and is likely transient and not indicative or the cause of tissue damage. In addition, glands of non-infected quail occasionally were dilated, as this can also occur with normal gland function (i.e., tear secretion). The lack of inflammation associated with *O. petrowi* is in contrast to studies of infected bobwhite in Texas (Andrea Bruno et al., 2015; Brym et al., 2018b, 2018a; Dunham, Bruno, et al., 2016; Dunham, Reed, et al., 2016; Villarreal et al., 2012). This may represent differing interpretations of the presence of leukocytes (white blood cells), of which some types (i.e., plasma cells) normally circulate in the Harderian gland, as they contribute to adaptive immune response, another function of this gland (Tahseen Abdul-Aziz et al., 2016). Of note, plasma cells were also observed in Harderian glands of bobwhite that lacked *O. petrowi* infections in our study. Thus, we considered the presence of inflammatory cells in this gland as normal. Also, the lack of lesions observed in ocular and periocular tissues of infected bobwhite in our study could reflect the much lower worm intensities we observed compared to previous studies in Texas (Andrea Bruno et al., 2015; Brym et al., 2018a; Dunham, Peper, et al., 2017; Dunham, Reed, et al., 2016). In Texas, it has been suggested that *O. petrowi* is one of the most significant parasitic contributors to the decline of the bobwhite population, so continued surveillance for and investigations into factors associated with transmission should be conducted (Brym et al., 2018b, 2018a; Dunham, Bruno, et al., 2016; Dunham, Reed, et al., 2016; Villarreal et al., 2012, 2016).

Research that seeks to contribute to the assessment of the population health of free-ranging wildlife species can provide critical information for selecting the best management strategies. Knowledge on the diversity and prevalence of parasites creates a base for understanding the potential health risks to bobwhites. In this study we found that free-ranging bobwhite sampled in western Oklahoma were in good health based on weights and fat stores, and had an overall low diversity and prevalence of parasites. However, several parasites (*A. pennula* and *O. petrowi*) detected have been associated with health impacts or are reported to be a concern (A. Bruno et al., 2019; Herzog et al., 2021; Villarreal et al., 2016), so they should be further investigated as potential population health risks to assist in future disease surveillance and management strategies pertaining to bobwhite.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

Overall, we found that bobwhites collected in nine wildlife management areas in western Oklahoma were in good nutritional condition and good apparent overall health status. Male and female bobwhite had similar weights and ample muscle mass with visible subcutaneous and visceral fat stores. These findings suggest that the bobwhite had access to sufficient food. Further, no significant gross or microscopic lesions were observed, further supporting the notion that bobwhite in western Oklahoma are in general good health with access to adequate resources.

Eyeworms (*O. petrowi*) have been recently considered to represent a conservation concern for bobwhites. In our study, eyeworms were detected in relatively low prevalence and mean intensity. Although Bruno et al. (2015) reported microscopic lesions (e.g,. inflammation) associated with *O. petrowi* infection, we observed absent to minimal inflammatory cell infiltrates in the Harderian gland and no signs of corneal ulceration or other ocular/periocular lesions. Thus, in our study, it does not appear that *O. petrowi* is affecting eye function or overall health of bobwhite in western Oklahoma, although should conditions change and prevalence and/or intensity increase, further assessment would be indicated.

Numerous external parasite species were detected but all were native parasites that are normally found on quail. Although ectoparasites generally occurred at high prevalences, the intensities were low and thus they most likely have little to no effect on bobwhite health. However, several tick species detected are vectors of important pathogens for animal and human health. *Aulonocephalus pennula* (caecal worm) was the most prevalent gastrointestinal parasite although their mean intensity and prevalence were relatively low compared to other studies in the Rolling Plains (Henry et al., 2017). We found that bobwhite with burdens >200 caecal worms had significantly less visible fat stores than bobwhite with lower caecal worm burdens. Thus, high caecal worm loads can have potential health impacts to individual bobwhite resulting in reduced survivability. We observed *Raillietina* sp. (tapeworms) primarily in juvenile bobwhite and these infected juvenile bobwhite had significantly less fat stores than juvenile bobwhite without tapeworms. Although more data are needed, this suggests that tapeworms may have potential health impacts on juvenile bobwhite. Two species of intramuscular parasites were detected, *Physaloptera* sp. and *Sarcocystis* sp. Both occurred in low prevalence and are unlikely to have population level impacts, but *Physaloptera* in muscles may escape from predators if intensities and associated muscle damage are extensive.

Numerous pathogens (e.g., viral, bacteria, protozoan) that can cause disease in various game bird and waterfowl species were not detected in bobwhite, and those pathogens that were detected were generally at a low prevalence. Among these, avian adenovirus, the causative agent of quail bronchitis and *Toxoplasma gondii*, a protozoan parasite, are a potential risk to quail health and further monitoring is warranted. However, overall, the prevalence and diversity of these selected pathogens in bobwhite in western Oklahoma were low and thus these are not likely to be a population risk.

In conclusion, bobwhite sampled in this study were infected with a low diversity of parasites and pathogens. It is important to note that we sampled wild-caught, presumably healthy individuals and no young chicks were sampled. Continued surveillance of *O. petrowi, A. pennula*, *Raillietina* spp., *T. gondii,* and avian adenovirus is warranted to determine long-term changes in prevalence and burdens that may occur and what impact any increases may have on regional and more widespread bobwhite population health. Future studies on other potential factors related to population decline coupled with pathogen surveillance would be informative because changes in environmental factors such as climate and habitat can also impact pathogen transmission and disease dynamics. While bobwhite in western Oklahoma are seemingly able to tolerate the current levels of parasitism and pathogens, the external pressures, including those that are anthropogenic, could alter host-pathogen-environment dynamics and thus disease risk to bobwhite.

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