

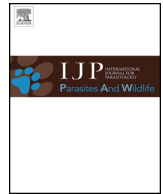


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**PREDICTING SEASONAL INFECTION OF EYEWORM (*OXYSPIRURA PETROWI*) AND CAECAL WORM (*AULONOCEPHALUS PENNULA*) IN NORTHERN BOBWHITE QUAIL (*COLINUS VIRGINIANUS*) OF THE ROLLING PLAINS ECOREGION OF TEXAS, USA**

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# Predicting seasonal infection of eyeworm (*Oxyspirura petrowi*) and caecal worm (*Aulonocephalus pennula*) in northern bobwhite quail (*Colinus virginianus*) of the Rolling Plains Ecoregion of Texas, USA

Kendall R. Blanchard<sup>a</sup>, Aravindan Kalyanasundaram<sup>a</sup>, Cassandra Henry<sup>a</sup>, Matthew Z. Brym<sup>a</sup>, James G. Surles<sup>b</sup>, Ronald J. Kendall<sup>a,\*</sup>

<sup>a</sup> The Wildlife Toxicology Laboratory, Texas Tech University, P.O. Box 43290, Lubbock, TX, 79409, USA

<sup>b</sup> The Department of Mathematics and Statistics, P.O. Box 41042, Texas Tech University, Lubbock, TX, 79409, USA

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## ABSTRACT

The northern bobwhite quail (*Colinus virginianus*) is a popular gamebird in the Rolling Plains Ecoregion of West Texas. However, there has been a population decline in this area over recent decades. Consistent reports indicate a high prevalence of the eyeworm (*Oxyspirura petrowi*) and caecal worm (*Aulonocephalus pennula*), which may be of major influence on the bobwhite population. While research has suggested pathological consequences and genetic relatedness to other pathologically significant parasites, little is known about the influence of climate on these parasites. In this study, we examined whether seasonal temperature and precipitation influences the intensity of these parasites in bobwhite. We also analyzed quantitative PCR results for bobwhite feces and cloacal swabs against temperature and precipitation to identify climatic impacts on parasite reproduction in this region. Multiple linear regression analyses were used for parasite intensity investigation while binary logistic regression analyses were used for parasite reproduction studies. Our analyses suggest that caecal worm intensity, caecal worm reproduction, and eyeworm reproduction are influenced by temperature and precipitation. Temperature data was collected 15, 30, and 60 days prior to the date of collection of individual bobwhite and compared to qPCR results to generate a temperature range that may influence future eyeworm reproduction. This is the first preliminary study investigating climatic influences with predictive statistics on eyeworm and caecal worm infection of northern bobwhite in the Rolling Plains.

## 1. Introduction

The northern bobwhite quail (*Colinus virginianus*; hereafter bobwhite), a popular gamebird in the United States of America has been experiencing a decline of > 4% per year (Sauer et al., 2013). This decline remains apparent despite their typical 5-year “boom and bust” cycle patterns (Hernández et al., 2007; Lusk et al., 2007). Much of this decline has impacted the Rolling Plains Ecoregion of Texas, a location considered as one of the last strongholds of bobwhite (Dunham and Kendall, 2017). Reasons for the decline are relatively unclear, but habitat loss, habitat fragmentation, weather variations, and land-use have previously been believed to be major influences (Rollins, 2007; Hernández et al., 2013). To investigate other contributors, a massive collaborative effort ensued to reevaluate the role of disease, contaminants, and parasites in these quail populations in the Rolling Plains. During this collaboration and ongoing survey work by the Wildlife

Toxicology Laboratory, the eyeworm (*Oxyspirura petrowi*) and caecal worm (*Aulonocephalus pennula*) were identified in high prevalence throughout this ecoregion with 100% infection in at least one area of the Rolling Plains (Henry et al., 2017).

The heteroxenous eyeworm is typically found under the nictitating membrane, lacrimal ducts and glands, and the orbital cavity of the bobwhite (Saunders, 1935; Addison and Anderson, 1969; Robel et al., 2003; Dunham et al., 2014; Bruno et al., 2015; Dunham and Kendall, 2017). Pathological investigations by Dunham et al. (2016a) and Bruno et al. (2015) found inflammation in the lacrimal duct and lesions on the Harderian gland. This is of great concern when considering these two tissues' importance to saturation of the eye (Holly and Lemp, 1977) and immune response (Payne, 1994), respectively. Recent phylogenetic analyses performed by Kalyanasundaram et al. (2018a) revealed its close relation to the human eyeworm, *Loa loa*, and the human and carnivore eyeworm, *Thelazia callipaeda*, which are both responsible for

\* Corresponding author. Wildlife Toxicology Laboratory, P.O. Box 43290, Texas Tech University, Lubbock, TX, USA.

E-mail address: [ron.kendall@ttu.edu](mailto:ron.kendall@ttu.edu) (R.J. Kendall).

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vision impairment and inflammation in their hosts (Barua et al., 2005; Nayak et al., 2016). Through PCR techniques developed by Almas et al. (2018), the differential grasshopper (*Melanoplus differentialis*) was identified as the primary carrier of the eyeworm though the infective larva can be carried in a variety of grasshopper species.

First described by Chandler (1935), the caecal worm is a free-floating, heteroxenous nematode of the caecum. Pathological analysis performed by Dunham et al. (2017a) revealed that infected bobwhite had reduced digesta available for nutrient absorption throughout the caecum. This is concerning as the caecum is an essential organ for water and nitrogen absorption, as well as immune response (Clench and Mathias, 1995). Dunham et al. (2017a) also speculate that because of their head shape and mouth parts (Inglis, 1958), higher infections encourage caecal worm attachment to the caecum walls. Phylogenetic analyses on the caecal worm also reveal a close relation to the family Anisakidae and Ascarididae, which contain species responsible for reduced energy levels, weight loss, and death in their hosts (Kalyanasundaram et al., 2017). Similar to the eyeworm, the caecal worm also uses an insect intermediate host in its lifecycle which was identified as most grass-eating grasshopper species of the Rolling Plains (Henry et al., 2018).

While experiments and data collection are continuing in an effort to understand morbidity and mortality associated with these parasites (Henry et al., 2017; Brym et al., 2018), further research on the role of climate factors should also be heavily considered. The relationship between climate and parasites has been of great interest over the last several decades because of its effects on parasite transmission (Dobson and Carper, 1992), larval development (Cattadori et al., 2005), intermediate host abundance (Dunham et al., 2017b; Henry et al., 2018), host populations (Hudson et al., 1998; Redpath et al., 2006), and arrested development (Armour and Bruce, 1974; Michel et al., 1976, 1978), among other effects. Speculations have been made by several researchers in the past about climatic effects on caecal worm and eyeworm. Lehmann (1984) suggests that the caecal worm thrives in drought conditions. Speculations by Dunham et al. (2017b) suggest that an increase in precipitation coincides with an increase in infection of both parasites throughout the Rolling Plains because of a higher abundance of insect intermediate hosts. Similarly, Henry et al. (2018) suggests that climate change also influences insect intermediate host populations and therefore, caecal worm transmission among bobwhite. Yet, there are few studies centered on climatic effects on these species of parasites.

Quantitative PCR (qPCR) can be used to indicate parasite reproduction based on parasite egg presence in fecal matter (Kalyanasundaram et al., 2018b) whereas necropsies can provide worm counts for parasite intensity. In this study, we use fecal samples, cloacal swabs, and average worm counts per bobwhite collected between March and October of 2014 through 2017 to perform predictive analyses on parasite reproduction and intensity in relation to temperature and precipitation. Our research objectives include (1) identify trends between temperature and precipitation in eyeworm and caecal worm reproduction and intensity using qPCR and necropsy methods, and (2)

forecast potential infection spread based on these parasites' reproduction from qPCR results in relation to temperature and precipitation.

## 2. Materials and methods

### 2.1. Ethics statement

All quail were trapped and handled accorded to Texas Parks and Wildlife permit SRP-1098-984 and SPR-0715-095 and Texas Tech University Animal Care and Use protocol 13066-08 and 16071-08.

### 2.2. Study area and sample collection

Bobwhite were collected between March and October of 2014–2017 from a 12,000ha privately owned cattle ranch in Mitchell County, Texas. Walk-in double funnel traps (91.4 × 60.9 × 20 cm) were baited with milo (*Sorghum bicolor*), covered with vegetation, and monitored two times per day to capture bobwhite as described in Dunham et al. (2014). All bobwhite individuals were transported to The Institute of Environmental and Human Health (TIEHH) aviary at Texas Tech where they were weighed, sexed, aged, cloacal swabbed, and a feces sample collected (Dunham et al., 2017b). Cloacal swabs and feces were collected upon capture and daily until necropsy to assess the reproductive activity of eyeworm and caecal worm based on presence of parasite eggs via quantitative PCR. For this study, samples collected on or within 1–2 days of capture were used in the analyses.

### 2.3. Necropsy and parasite collection

Euthanasia and necropsies were performed as described in Dunham et al. (2014). Eyeworms were extracted from the eyes by removing the eyeball and associated tissues into a petri dish as outlined in Dunham et al. (2016a). Caecal worm collection followed protocols described in Dunham et al. (2017b) where caeca were removed and placed in a 20-mesh sieve, then cut into smaller sections to assess caecal worm count.

### 2.4. DNA extraction and qPCR

Feces samples weighing 0.18–0.22 g and cloacal swabs were snap-frozen in liquid nitrogen and then extracted using the QIAamp DNA Stool Mini Kit (Germany) following manufacturer's protocol as outlined in Kistler et al. (2016a) with a final elution step of 50 µL per sample followed from Kalyanasundaram et al. (2018b). Quantitative PCR techniques also followed Kalyanasundaram et al. (2018b) methods with 20 µL mastermix volumes as follows: 10 µL Taqman Fast Advanced Mastermix (Applied Biosystems), 0.4 µL of forward and reverse eyeworm primers and eyeworm probe, 0.2 µL of forward and reverse caecal worm primers and caecal worm probe, 0.2 µL of forward and reverse quail primers and quail probe, 0.1 µL bovine serum albumin (BSA), and 7.66 µL of molecular-grade water. Primer and probe sequences used in this study are outlined in Table 1.

**Table 1**

Primer and probe sequences used in qPCR methods.

Primer/Probe	Sequence	Target	Product Size
Oxy2448F	GTTTCCTCATGTGATTTTCATTTTGT	Eyeworm ITS2 (Kistler et al., 2016a)	149bp
Oxy2597R	ATAAACGGTTATTGTTGCCATATGCT		
Oxy_Probe_1	FAM-AAAGAAAGGTAATTCATCTGGT-MGB		
Apen F1	GGGTTGTGGTACTAGGTGGGT	Caecal Worm COX1 (Kalyanasundaram et al., 2018b)	120bp
Apen R1	GCACCCAAAATAGAACTCACCCC		
Apen_Probe_1	VIC-GGTCATCCTGGTAGAAGCGTTG-MGB		
ND2_70F	CAACCACTGAATCATAGCCTGAAC	Northern Bobwhite NADH2 (Kistler et al., 2016a)	79bp
ND2_149R	GGTGGTGGGATTTTGAAATGAG		
Quail_ND2_Probe1	NED-AGGAACCAATCAC-MGB		

## 2.5. Data analysis

Daily mean temperature and precipitation data was accessed for Sterling City, TX (31°50'4.92" N, –100°58'57.72"W) from the [National Oceanic and Atmospheric Administration \(NOAA\) \(2018\)](#). This site was chosen because of its proximity to the study area and the consistency in which temperature and precipitation data was collected by the selected weather station. Temperature and precipitation values were converted to metric units and then grouped and averaged by season and year from 2014 to 2017 as spring (March–May), summer (June–August), and fall (September–October). Eyeworm and caecal worm counts among bobwhite between 2014 and 2017 were grouped and averaged in the same manner. The seasonally averaged data for temperature, precipitation, and worm counts were compared to better understand climatic influences on eyeworm and caecal worm intensities in bobwhite. Positive qPCR results per individual bobwhite were also totaled and then grouped by season and year. These results were then compared with seasonally averaged temperature and precipitation to identify if climatic factors influence infection spread to intermediate hosts using parasite reproduction as an indicator. A total of 244 individual bobwhite were assessed for average eyeworm counts, 268 for average caecal worm counts, and 141 cloacal swabs and feces from individual bobwhite were used for analysis by qPCR. This dataset was not robust enough to support demographic comparisons and thus, was not analyzed in this study. Summary data for averaged climatic variables, average worm counts, and qPCR results are visualized in [Table 2](#).

For average worm count analyses, multiple linear regression analyses were run to study relationships between the average counts per bobwhite with average seasonal temperature, precipitation, and the interaction between average seasonal temperature and precipitation. Because of small sample sizes, these analyses were weighted by the number of samples for both eyeworm and caecal worm. For seasonally totaled qPCR positive results, a binary logistic regression analysis was run to examine relationships between parasite reproduction with seasonally averaged temperature, precipitation, and the interaction between temperature and precipitation.

In addition, temperature data was also used to predict eyeworm and caecal worm infection spread using positive and negative qPCR results. To do this, temperature data from NOAA was grouped into 15, 30, and 60 days prior to individual bobwhite collection date and then compared to their corresponding qPCR results, positive or negative. Because of the lack of variability in precipitation data (with 83% of analyzed data having < 1 cm of precipitation) and thus, subsequently large confidence intervals, precipitation was not used in predicting parasite reproduction in this study. For these analyses, binary logistic regressions

with quadratic terms were used to assess significance between qPCR results and the temperature data while also identifying an optimal temperature range which may facilitate future parasite reproduction and infection spread. Statistical significance was based on  $p < 0.05$ . All statistical analyses were run in Minitab (v8) and all data was tested for normality during regression analyses.

## 3. Results

### 3.1. Eyeworm intensity and reproduction

The multiple linear regression analysis for average eyeworm count with temperature and precipitation had no statistical significance. For the binary logistic regression analysis of positive qPCR results, each variable was run individually because of the heavy collinearity between temperature and precipitation. In these individual tests, both precipitation and temperature were statistically significant. The trend exemplified in [Fig. 1d](#) indicates that detection of eyeworm reproduction in bobwhite increases with increasing temperature and precipitation.

### 3.2. Caecal worm intensity and reproduction

The multiple linear regression analysis for average caecal worm count for individual bobwhite with seasonally averaged temperature and precipitation had a marginal significance ( $p = 0.07$ ). The general trend seen in this analysis reveals higher caecal worm intensities in lower temperatures and lower precipitation ([Fig. 1a](#)).

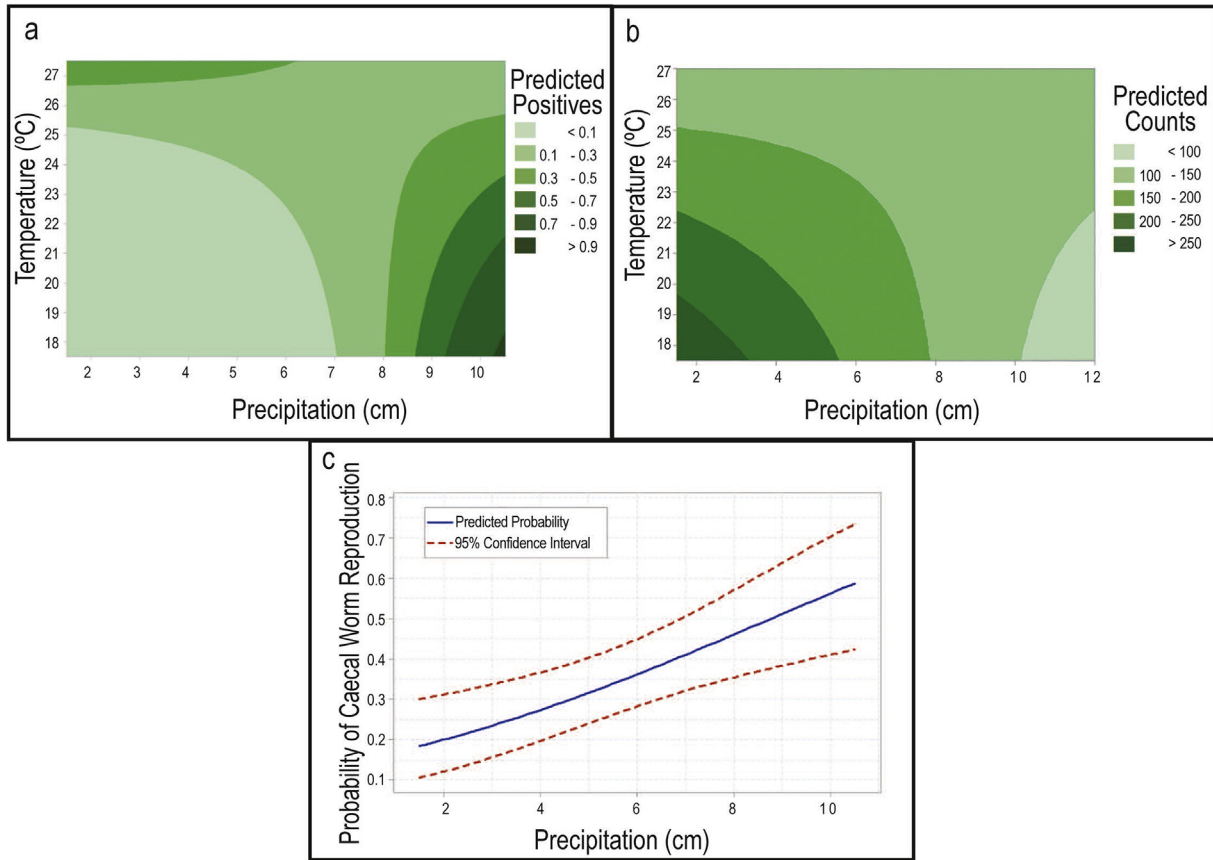
Because of a heavy collinearity between temperature and precipitation in the binary regression analysis with positive qPCR results, each variable was run individually against positive qPCR results using separate binary logistic regressions. In these analyses, seasonally averaged temperature was not significant whereas seasonally averaged precipitation was significant. The predicted output of positive qPCR results indicates that as precipitation increases, the probability of reproduction of caecal worm in birds increases as well ([Fig. 1b](#)).

### 3.3. Climatic influence on parasite reproduction

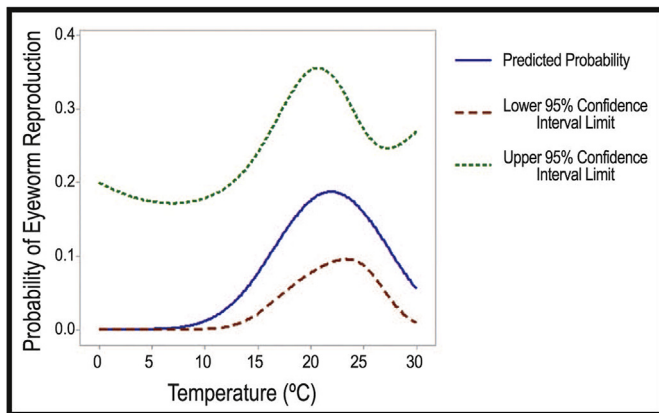
Because of the lack of variability in precipitation data, caecal worm reproduction was not analyzed because of the lack of significance between caecal worm reproduction and temperature. Eyeworm reproduction, however, was significant with temperature. When qPCR results were compared to temperatures of collection dates, the temperature 60 days prior to collection date was marginally significant ( $p = 0.07$ ), and temperature 15 and 30 days prior to the collection date

**Table 2**  
Data on all sample sizes used in statistical analyses of average worm counts and qPCR positives.

Season and Year	Climatic Variables		Total Positive qPCR Results		Average Worm Counts	
	Temperature (°C)	Precipitation (cm)	Eyeworm	Caecal worm	Eyeworm	Caecal worm
<b>2014</b>						
Spring	20.00	7.53	0 (N = 0)	0 (N = 0)	23.5 (N = 14)	139.0 (N = 14)
Summer	26.94	1.49	1 (N = 11)	2 (N = 11)	33.0 (N = 16)	149.3 (N = 16)
Fall	22.08	4.36	2 (N = 19)	1 (N = 19)	16.0 (N = 21)	91.5 (N = 21)
<b>2015</b>						
Spring	19.31	7.01	1 (N = 2)	1 (N = 2)	26.0 (N = 18)	181.0 (N = 18)
Summer	26.66	3.49	6 (N = 7)	6 (N = 7)	22.0 (N = 39)	101.0 (N = 39)
Fall	22.36	10.22	14 (N = 22)	20 (N = 22)	23.5 (N = 27)	74.5 (N = 51)
<b>2016</b>						
Spring	17.59	6.68	0 (N = 20)	3 (N = 20)	13.0 (N = 27)	162.3 (N = 27)
Summer	27.22	4.90	1 (N = 4)	0 (N = 4)	18.5 (N = 10)	238.5 (N = 10)
Fall	22.50	6.70	0 (N = 8)	1 (N = 8)	19.5 (N = 16)	180.0 (N = 16)
<b>2017</b>						
Spring	18.70	1.85	0 (N = 21)	8 (N = 21)	18.0 (N = 26)	277.6 (N = 26)
Summer	26.48	9.76	1 (N = 10)	3 (N = 10)	10.6 (N = 14)	153.3 (N = 14)
Fall	20.83	3.49	0 (N = 17)	4 (N = 17)	16.0 (N = 16)	154.5 (N = 16)



**Fig. 1.** Contour and scatterplot of relationships between temperature and precipitation on parasite worm burdens and egg shedding. a) Predicted caecal worm intensity against temperature and precipitation contour plot. b) Scatterplot of predicted caecal worm reproduction against precipitation. d) Predicted eyeworm reproduction against temperature and precipitation contour plot.



**Fig. 2.** Scatterplot of predicted eyeworm reproduction with temperature 60 days prior to collection date with upper and lower 95% confidence intervals.

were not significant. This test also determined that temperatures between approximately 15 °C and 26 °C 60 days prior to collection of bobwhite may facilitate increased probability of future eyeworm reproduction and therefore, infection spread to intermediate hosts. These results are illustrated in Fig. 2.

**4. Discussion**

While there are numerous recent studies on eyeworm and caecal worm prevalence in the Rolling Plains (Dunham et al., 2016b; Henry et al., 2017; Brym et al., 2018; Bruno et al., 2018), this is the first study

to analyze and predict eyeworm and caecal worm intensity and reproduction in bobwhites based on climatic factors. By analyzing predictive statistics on available temperature and precipitation data, this study reveals the potential of climate to influence both eyeworms and caecal worms. Understanding how seasonal dynamics and changing climatic patterns influence these parasites is an essential step in analyzing how these infections alter with global warming (Altizer et al., 2006). This is essential in the Rolling Plains ecoregion because the average temperature in the Rolling Plains is predicted to increase from 35.5 °C to 38.4 °C in July, -1.1 °C–2.1 °C in January, and annual rainfall may decrease from 50 cm/year to as low as 37.3 cm/year (Modala et al., 2017; Texas A&M AgriLife Extension, 2018).

With the predicted climate changes in the Rolling Plains, this study is useful for understanding how climate can potentially influence eyeworm and caecal worm infection dynamics. For example, results from this study indicate temperature 60 days prior to collection date of bobwhite may influence eyeworm reproduction which coincides with Kistler et al.’s (2016b) experimental infection indicating eyeworm reproduction in bobwhite 52–56 days post infection. Similarly, caecal worm intensities increase in low precipitation which supports Lehmann’s (1984) theory that caecal worm infection is exacerbated in drought conditions. However, this study analyzes what is likely to be a few factors in the multitude of influences that dictate eyeworm and caecal worm infection dynamics. Despite this, the current data set reveals preliminary insight as to the potential roles of temperature and precipitation, especially when compared to other parasite species.

Climate effects on parasites have been well documented, particularly regarding their effects on parasite development, reproduction, and intensity (Schad, 1977; Lima, 1998; Sommerville and Davey, 2002; Sissay et al., 2007). Such infection dynamics are apparent in other

nematodes like *Ostertagia ostertagi*, a gastrointestinal parasite of cattle, which has been shown to enter stages of arrested development during the winter seasons (Michel et al., 1974, 1975; 1976). Temperature can also induce arrested development for the larvae of *Obeliscoides cuniculi*, a parasite of rabbits, in which larvae stored at 4 °C were dormant while those kept at 15 °C remained active (Fernando et al., 1971). A similar instance has been identified for both eyeworms and caecal worms in the Rolling Plains where there was less detection of parasite eggs in feces collected during September and October (Kalyanasundaram et al., 2018b), which may have been because of an arrested development state caused by lower temperatures. Furthermore, a study by Lima (1998) examining gastrointestinal nematode infection of *Cooperia* spp. in Nellore cattle of Brazil found that cattle had the lowest worm counts during July in the dry season when compared to the rainy season. Contrastingly, Dunham et al. (2016b) observed a decrease in eyeworm and caecal worm prevalence with decreased precipitation.

In addition to the direct effects of temperature and precipitation, caecal worm and eyeworm infection dynamics may be influenced by the abundance of insect intermediate hosts. For instance, Henry et al. (2017) speculated that the parasite-induced die-off of bobwhite in Mitchell County, TX during 2017 may have been because of increased rainfall and a resulting surge in intermediate host populations. This trend has been demonstrated before in some insect species having greater density of adult species in moist habitats (Janzen and Schoener, 1968). Because reproduction in both the eyeworm and caecal worm increases with increasing precipitation, this may indicate a relationship between insect intermediate host and parasite reproduction to maintain the parasite life cycle. Additionally, parasite egg viability is higher in moist conditions (Rogers and Sommerville, 1963; Gaasenbeek and Borgsteede, 1998), which may also increase the advantages of parasite reproduction at that time and therefore, increase the likelihood of successful transmission to intermediate hosts.

Moreover, climate change may facilitate expansion of arthropod intermediate host ranges (Guo et al., 2009) and can extend native ranges of various parasites as formerly uninhabitable areas become suitable (Lafferty, 2009). This was also suspected by Henry et al. (2018) who postulated that caecal worm infections might spread geographically in response to rising temperatures. Climate-induced expansions in either arthropod or parasite ranges could also lead to the possibility of host switching, as potentially new host species become exposed (Brooks and Hoberg, 2007). The increase in extreme climatic events associated with global climate change (Coumou and Rahmstorf, 2012) have also been linked to the simultaneous emergence of parasites from arrested development, resulting in epizootic events (Hudson et al., 2006).

Understanding climatic factors of importance that trigger these epizootic events may also prove to be important for the timing of anthelmintic treatment. This is because anthelmintics are not as likely to be as effective during periods of arrested development because of the lower energy requirements of nematodes (Sommerville and Davey, 2002). For instance, timing of anthelmintic treatment is suggested to contribute to the lack of response in *O. oestertagi* (Pritchard et al., 1978) and most anthelmintics are ineffective against arrested larvae of parasites that infect horses (Proudman and Matthews, 2000). Since growth and development are halted during arrested development, is it also likely that reproduction in parasites is also not occurring (Blitz and Gibbs, 1972; Gibbs, 1986). Thus, understanding that temperatures between 15 °C and 26 °C may influence eyeworm reproduction 60 days later could allow implementation of anthelmintics when they would be most effective.

While this study provides preliminary results for eyeworm and caecal worm dynamics in the Rolling Plains, further investigation is needed to better understand the influence of climatic variables on these parasites. Studies have revealed that eyeworm and caecal worm prevalence can vary seasonally and spatially in the Rolling Plains. For example, Dunham et al. (2016b) reports that bobwhite trapped between

August and October of 2011–2013 had an eyeworm prevalence of 41% while Bruno (2014) found a 66% prevalence in bobwhite collected during the 2012 to 2013 hunting season (November–February). Yet, Henry et al. (2017) reported 100% prevalence of eyeworms in bobwhite captured in March 2017 from Mitchell County, Texas. In contrast, caecal worm prevalence has remained at 90%–100% in the Rolling Plains over the past several years, though intensity can vary by individual bobwhite (Dunham et al., 2017b; Brym et al., 2018; Bruno et al., 2018). These differences in prevalence could be explained by variances in temperature and precipitation as environmental conditions may vary from county to county in the Rolling Plains (Modala et al., 2017), potentially impacting intensities of both parasites.

Further studies are also necessary as the current results cannot conclude whether temperature or precipitation have an influence on eyeworm intensity. Additionally, the low predicted probability in caecal worm reproduction with climate (Fig. 1b) and eyeworm reproduction 60 days prior to bobwhite collection dates (Fig. 2) introduce uncertainty in these predictions. Furthermore, marginal significance was observed in a few analyses. However, a larger data set with samples collected from multiple locations in the Rolling Plains would help account for these variable factors and increase the accuracy of these predictions, thus decreasing uncertainty while also reducing time and resources required for laboratory and field testing of infection. For example, month-by-month variations of parasite egg presence have been presented for other parasites (Wood et al., 2013), indicating that a more robust dataset may further the understanding of climatic variables on eyeworm and caecal worm reproduction. Similarly, while there have been a few studies expanding on mean parasite abundance and bobwhite demographics (Dunham et al., 2014; Dunham et al., 2017b), a larger dataset may allow future analyses to include parasite reproduction in relation to demographic trends. Nevertheless, because of limitations in the current data, it is suggested that consistent testing should continue to better analyze eyeworm and caecal worm dynamics in bobwhite hosts.

In conclusion, this was the first predictive study on the eyeworm and caecal worm infecting bobwhite of the Rolling Plains Ecoregion. Results suggest climate influences caecal worm intensity, caecal worm reproduction, and eyeworm reproduction which provides preliminary insight to possible infection dynamics based on temperature and precipitation. Furthermore, an estimated time range and temperature range were generated based on eyeworm reproduction to suggest timing in which anthelmintic treatment will be most effective to mitigate the eyeworm. However, the current dataset could not provide conclusive evidence on all aspects of infection dynamics that were targeted in this study. For optimization of future predictive analyses, larger sample sizes and continuous, thorough monitoring should be used in the future to determine more conclusive effects climate has on these parasites.

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