International Journal for Parasitology: Parasites and Wildlife 25 (2024) 101006

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Northern bobwhite *(Colinus virginianus)* population response to anthelminthic treatment in the Rolling Plains ecoregion of Texas, 2014–2016

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ARTICLE INFO

Keywords:
Northern bobwhite
Colinus virginianus
Anthelmintics
Quail
Helminths
Supplemental feed

ABSTRACT

Northern bobwhite quail (*Colinus virginianus*) are an economically significant gamebird that has experienced continued general decline in the Rolling Plains ecoregion of Texas. Habitat loss and changing environmental conditions have been cited as major contributors to this decline, with factors such as parasites being considered inconsequential. To better assess the impacts of parasite infections on bobwhite populations in the Rolling Plains, bobwhite abundance was monitored in response to anthelminthic treatment. With the prevalence of *Oxyspirura petrowi* and *Aulonocephalus pennula* infections in quail from Mitchell County, Texas confirmed by previous studies, the anthelminthic agent Fenbendazole was introduced as a means of parasite control in 2014–2015. Bobwhite abundance was determined through a series of call counts which provided an index of bobwhite populations and were conducted throughout the course of the study. In 2016, call counts revealed a significant increase of bobwhite in the area subject to Fenbendazole treatment, while untreated areas showed no changes in abundance. Fall populations of bobwhite in the treated zone approached 300% of those in untreated areas, these findings suggest that parasites may have a more significant impact on quail populations in the Rolling Plains than previously suspected. With the importance of bobwhite as a game bird in the Rolling Plains, the potential impacts of parasites must be taken into consideration as a factor contributing to bobwhite declines. Further research into the long-term effects these parasites have on quail populations in the ecoregion may aid landowners in developing affordable and effective conservation strategies.

1. Introduction

Northern bobwhite quail (*Colinus virginianus*) are a popular North American game bird that have been experiencing a widespread decline throughout their native range over the past several decades (Brennan 1991; Sauer et al., 2013). This trend extends into the Rolling Plains ecoregion of West Texas, an area that was once considered a stronghold for the species. The popularity of northern bobwhite among hunters and their economic significance to local communities (Johnson et al., 2012) have spurred significant efforts to determine the possible factors behind the observed decline in quail populations. Factors such as habitat loss and degradation, dynamic weather patterns, and changing agricultural practices have been cited as major contributors to the widespread decrease in bobwhite numbers (Brennan 1991; Bridges et al., 2001; Hernández et al., 2013). Additionally, the presence of diseases and parasites have been noted to adversely affect quail populations, although these factors have typically been undervalued (Peterson 2007). In 2011, a comprehensive effort was launched to address these understudied factors and focused on investigating the effects that disease caused by pathogens and contaminants have on quail in the Rolling Plains of Texas and Oklahoma. This initiative, known as Operation Idiopathic Decline (OID), entailed collaboration between several major universities and led to research that highlighted the prevalence of helminths in quail throughout the ecoregion.

A major finding of OID was evidence of the widespread occurrence and high levels of eyeworm (*Oxyspirura petrowi*) and cecal worm (*Aulonocephalus pennula*) infections in quail from the Rolling Plains (Dunham et al. 2016a, 2016b). These discoveries spurred further research into the effects parasites may have at both the individual and population level. Both nematodes are heteroxenous, using insects as an intermediate host (Almas et al., 2018; Henry et al. 2019, 2020). Eyeworms reside within the eyes and associated tissues of quail (Saunders

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https://doi.org/10.1016/j.ijppaw.2024.101006
Received 18 June 2024; Received in revised form 16 October 2024; Accepted 17 October 2024
Available online 20 October 2024

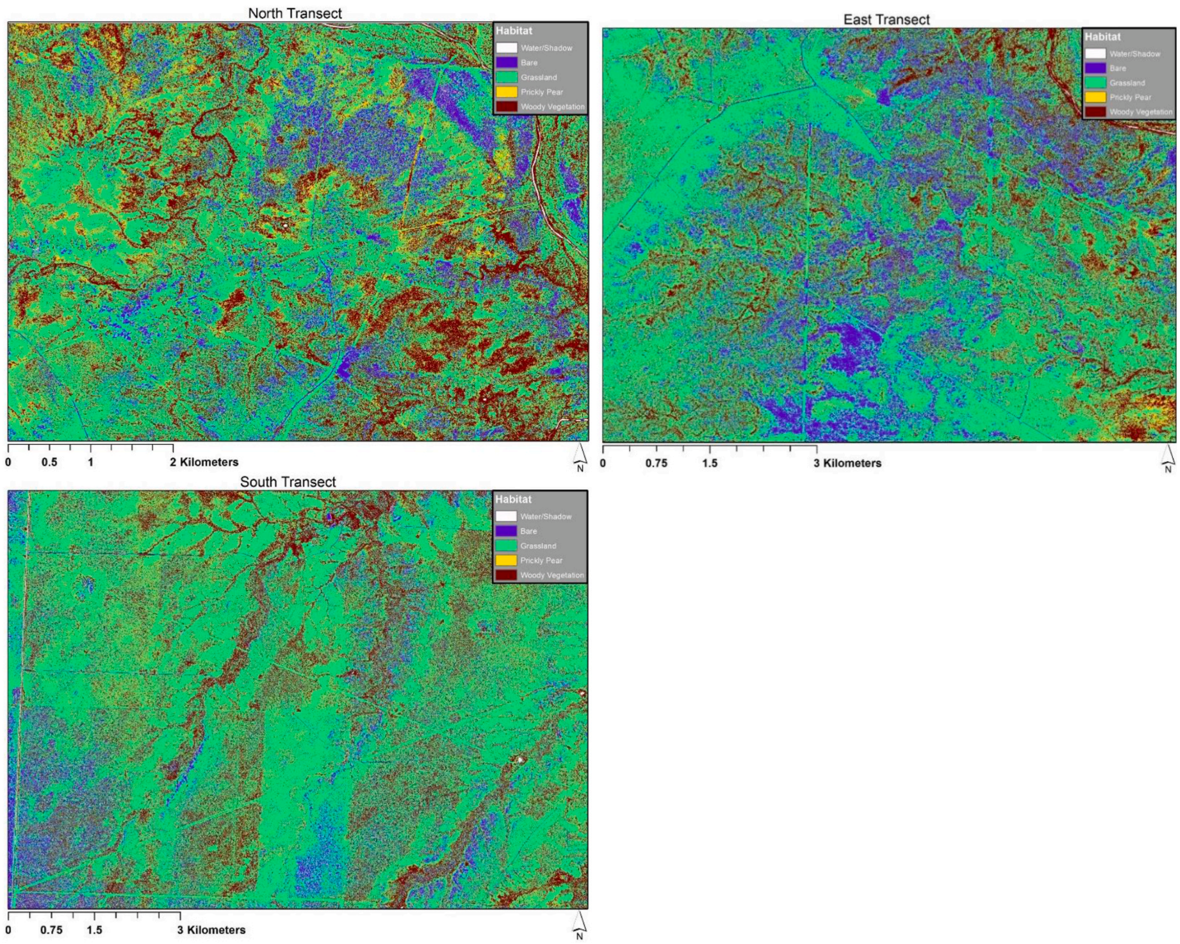

Fig. 1. Maps of habitat composition across all three transects.

1935; Addison and Anderson 1969; Dunham et al., 2014b; Bruno et al., 2015), while the cecal worm is an intestinal parasite that predominantly inhabits the ceca of the host (Peterson 2007). Research into the pathological ramifications of eyeworm infections has confirmed inflammation and damage to the cornea, eye ducts, and glands (Bruno et al., 2015; Dunham et al., 2016c). Although consequences of cecal worms in quail have yet to be determined, similar parasites have been known to cause inactivity, weight loss, and reduced growth in game birds (De Rosa and Shivaprasad 1999; Nagarajan et al., 2012). Additionally, a survey did find an increased probability of wild bobwhite having poor body condition when heavily infected with cecal worms (Wyckoff et al., 2023). These pathological infestations may increase the vulnerability of quail to predation and adverse environmental conditions, particularly when the prevalence of these parasites is high. Between 2011 and 2013, *O. petrowi* were documented in 29 counties in the Rolling Plains, with an outbreak of >90% infection rates being recorded in adult northern bobwhites in Mitchell County, Texas during the summer of 2013 (Dunham et al. 2014b, 2016b). This trend continued into 2014 and 2015, with >94% infections of *O. petrowi* and >97% infections of *A. pennula* in Mitchell County (Dunham et al., 2016a). Given the high prevalence and potential consequences of cecal and eyeworms in quail throughout the Rolling Plains, it is imperative to consider the effects these parasites may have on quail populations in this area.

Parasites have been documented as a factor regulating wildlife populations (Hudson et al., 1992, Tompkins et al., 2002), and it is plausible that they play a role in the population dynamics of northern bobwhite as well. An example of this interaction is the impact of the cecal nematode *Trichostrongylus tenuis*, on the abundance of another Galliform, the red grouse (*Lagopus lagopus scotica*). Infestations of *T. tenuis* have been found to reduce the breeding production of red grouse, as well as increasing their vulnerability to predation (Hudson 1986; Hudson et al., 1992). The effects of *T. tenuis* infection were of such significance, that the control of the parasites through an anthelminthic treatment prevented crashes in red grouse populations (Hudson et al., 1998). These findings confirmed the role of *T. tenuis* as a driving force in the population cycles of red grouse and, considering the high prevalence of *O. petrowi* and *A. pennula* in the Rolling Plains, it is feasible that a parasitic infection in this ecoregion could have a similar effect on bobwhite populations. Pathological consequences of *O. petrowi* and *A. pennula* may compound the effects of other stressors on bobwhite, thus jeopardizing their survival. Consequently, if a significant number of individuals within a population experienced attrition, the net result would be an overall decline in bobwhite abundance. Although the research to determine how parasite loads affect the fitness of individual bobwhites is still underway, an approach like the one used by Hudson et al. may illustrate how *O. petrowi* and *A. pennula* impact quail populations.

Hudson and his colleagues were able to assess the consequences of *T. tenuis* infection on red grouse abundance by monitoring grouse population response to the control of parasites. Using a similar method of anthelminthic treatment and population monitoring, we were able to compare the abundance of quail in treated vs untreated regions of Mitchell County, Texas. These comparisons allowed us to gauge the potential impacts that *O. petrowi* and *A. pennula* had on the survivability of quail in our study area. As we gather more information about the effects these parasites have on the fitness of individual quail, we continue to expand upon our understanding of the impact parasites may have on bobwhite populations in the Rolling Plains. Considering the

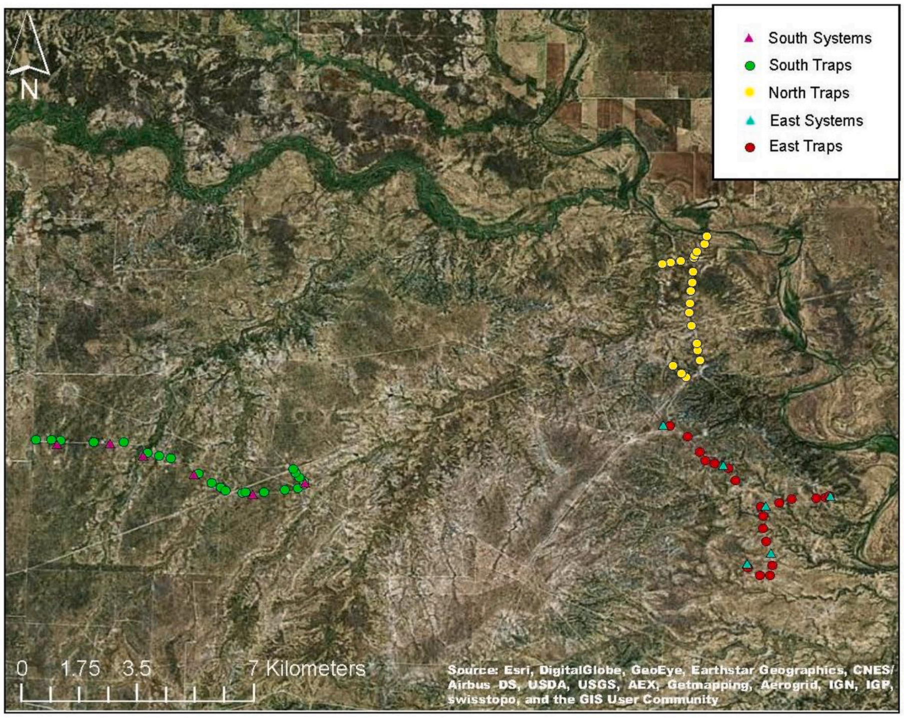

Fig. 2. Map of study area in Mitchell County, Texas showing trapping locations and treatment systems.

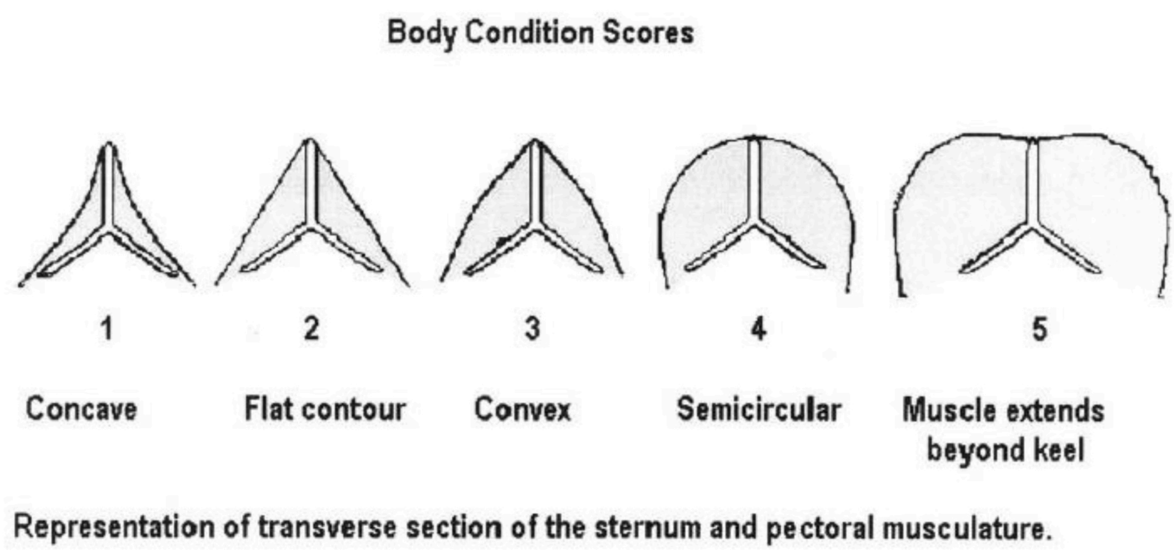

Fig. 3. Figure representing scale used to assess northern bobwhite condition score.

importance of bobwhite as a game bird in the region, the potential to mitigate trending declines in quail populations through the development of disease management strategies, such as parasite control, is of great value. With eyeworms having been documented in other species of birds, ranging from songbirds to lesser prairie chickens (*Tympanuchus pallidicinctus)* (Dunham and Kendall 2014, 2016; Dunham et al., 2014a), a better grasp of the role avian parasites play in the population dynamics of their hosts may benefit conservation efforts on a wider scale.

2. Methods

2.1. Ethics statement

Captured birds were handled according to Texas Parks and Wildlife research permits SPR-1098-984 and SPR-0715-095. This experiment was approved under Texas Tech University Animal Care and Use Committee protocol 16071-08 and complied with Institutional Animal Care and Use Committee guidelines.

2.2. Study area

The study area was consistent with that used by Dunham et al. (2014b, 2016a) and was conducted on the same 120,000-ha private ranch in Mitchell County, Texas. The study area was further divided into a north, east, and south transect to allow for the separation of treated and untreated areas, and all transects encompassed equivalent habitat to prevent habitat variability from influencing results (Fig. 1). All transects were in relative proximity to each other to ensure uniformity of

Table 1
Results of our covey counts for the 2015 and 2016 field seasons. *Averages and standard deviation calculated for all transects using source data.

		North	East	South	All*
2015 covey count *(16-18th October)*	Average number of covey calls per station (±SD)	4 ± 2	5 ± 2	8 ± 4	6 ± 3
	Average number of coveys flushed per route (±SD)	1 ± 1	1 ± 1	2 ± 2	1 ± 1
	Average covey size (±SD)	5±N/A	12 ± 11	11 ± 4	10 ± 5
	Total number of covey calls	11	15	24	50
	Total number of coveys flushed	1	2	6	9
2016 covey count *(15-17th October)*	Average number of covey calls per station (±SD)	6 ± 1	16 ± 2	6 ± 3	9 ± 5
	Average number of coveys flushed per route (±SD)	1 ± 1	2 ± 2	3 ± 2	2 ± 2
	Average covey size (±SD)	11 ± 2	11 ± 4	17 ± 8	14 ± 7
	Total number of covey calls	18	48	18	84
	Total number of coveys flushed	3	7	9	19

Table 2
P-values for Tukey post hoc for average covey call counts per station on all transects in 2015 and 2016.

	East 2015	North 2015	South 2015	East 2016	North 2016	South 2016
East 2015		0.9793	0.6371	*0.0010*	0.9943	0.9943
North 2015			0.2839	*0.0004*	0.8230	0.8230
South 2015				*0.0130*	0.8956	0.8956
East 2016					*0.0024*	*0.0024*
North 2016						1.000

Table 3
Results of our spring cock call counts for the 2015 and 2016 field seasons. *North transect has 6 listening stations as opposed to the 10 found on the other transects.

2016 call count *(6-8th June)*	Average number of males per station (±SD)	7 ± 2	9 ± 4	5 ± 1	7 ± 3
	Average number of calls per station (±SD)	84 ± 26	126 ± 30	63 ± 24	92 ± 38
	Average number of males per day	44	89	47	60
	Average number of calls per day	506	1257	634	799
	Total number of males	131	268	143	542
	Total number of calls	1519	3772	1902	7193

environmental and weather conditions but were separated by at least 2 km to prevent the overlap of birds (Fig. 2).

2.3. Treatment

From 2014 to 2015, the anthelminthic agent Fenbendazole was administered within the study area in late July or early August to assess the effects of *O. petrowi* and *A. pennula* infection on bobwhite

Table 4
P-values for Tukey post hoc for average daily spring cock call counts per transect on all transects in 2015 and 2016.

	East 2015	North 2015	South 2015	East 2016	North 2016	South 2016
East 2015		*<0.0001*	0.7587	*0.0064*	0.2219	0.3166
North 2015			*<0.0001*	*0.0048*	*<0.0001*	*<0.0001*
South 2015				*0.0008*	0.8786	*0.0386*
East 2016					*0.0002*	0.2269
North 2016						*0.0063*

		North*	East	South	All
2015 call count *(3-5th June)*	Average number of males per station (±SD)	3 ± 1	5 ± 2	6 ± 3	5 ± 2
	Average number of calls per station (±SD)	33 ± 20	45 ± 25	23 ± 16	34 ± 22
	Average number of males per day	16	50	55	40
	Average number of calls per day	197	445	234	292
	Total number of males	49	149	166	364
	Total number of calls	591	1335	702	2628

Table 5
Table showing the number of bobwhite trapped, total recaptures and number of individuals recaptured (in parentheses) during the 2015 and 2016 field seasons.

	Captures				Recaptures			
	North	East	South	Total	North	East	South	Total
2015	65	75	44	184	24 (17)	18 (12)	10 (8)	52 (37)
2016	70	53	79	202	32 (15)	14 (7)	31 (19)	77 (41)

Table 6
Table summarizing our radio-tracking results during the 2015 and 2016 field seasons.

		North	East	South	Total
2015	**Total number of collared bobwhites**	44	36	31	111
	Total number of mortalities	23	16	14	53
	Average number of birds monitored per session (±SD) *(4/17/2015–10/16/2015)*	33 (±8)	20 (±4)	19 (±3)	72 (±8)
	Average number of birds heard per session (±SD) *(4/17/2015–10/16/2015)*	18 (±10)	13 (±3)	9 (±6)	40 (±8)
	Average number of mortalities per session (±SD) *(4/17/2015–10/16/2015)*	1 (±2)	1 (±1)	1 (±1)	3 (±1)
2016	**Total number of collared bobwhites**	38	40	39	117
	Total number of mortalities	22	23	26	71
	Average number of birds monitored per session (±SD) *(3/21/2016–10/10/2016)*	25 (±6)	26 (±7)	25 (±8)	76 (±7)
	Average number of birds heard per session (±SD) *(3/21/2016–10/10/2016)*	24 (±7)	24 (±8)	22 (±10)	70 (±8)
	Average number of mortalities per session (±SD) *(3/21/2016–10/10/2016)*	1 (±1)	1 (±1)	1 (±1)	3 (±1)

Table 7
Mortality rates across north, south and east transects during regular telemetry monitoring period of 2016 field season.

Week	North			East			South			Total		
	Morts	Total	Mort %	Morts	Total	Mort %	Morts	Total	Mort %	Morts	Sum	Mort %
3/21	0	26	0	0	24	0	1	39	2.56	1	89	1.12
3/28	0	29	0	0	27	0	3	38	7.89	3	94	3.19
4/4	1	36	2.78	1	36	2.78	1	35	2.86	3	107	2.80
4/11	2	36	5.56	1	39	2.56	1	34	2.94	4	109	3.67
4/18	0	34	0	1	38	2.63	0	33	0	1	105	0.95
4/25	0	34	0	1	37	2.70	0	33	0	1	104	0.96
5/2	2	34	5.88	1	36	2.78	2	33	6.06	5	103	4.85
5/9	2	32	6.25	2	35	5.71	2	31	6.45	6	98	6.12
5/16	0	30	0	2	33	6.06	0	29	0	2	92	2.17
5/23	3	30	10.00	4	31	12.90	0	29	0	7	90	7.78
5/30	1	27	3.70	1	27	3.70	0	29	0	2	83	2.41
6/6	1	26	3.85	0	26	0	0	29	0	1	81	1.23
6/13	1	25	4.00	0	26	0	1	29	3.45	2	80	2.50
6/20	1	24	4.17	0	26	0	1	28	3.57	2	78	2.56
6/27	0	23	0	3	26	11.54	2	27	7.41	5	76	6.58
7/4	1	23	4.35	2	23	8.70	1	25	4.00	4	71	5.63
7/11	0	22	0	0	21	0.0	0	24	0	0	67	0
7/18	0	22	0	0	21	0.0	1	24	4.17	1	67	1.49
7/25	0	22	0	0	21	0.0	0	23	0	0	66	0
8/1	1	22	4.55	0	21	0.0	1	23	4.35	2	66	3.03
8/8	2	21	9.52	0	21	0.0	1	22	4.55	3	64	4.69
8/15	0	19	0.00	0	21	0.0	4	21	19.05	4	61	6.56
8/22	0	19	0.00	1	21	4.8	1	17	5.88	2	57	3.51
8/29	1	19	5.26	1	20	5.0	2	16	12.50	4	55	7.27
9/5	0	18	0.00	1	19	5.3	1	14	7.14	2	51	3.92
9/12	0	18	0.00	0	18	0.0	0	13	0.00	0	49	0.00
9/19	1	18	5.56	0	18	0.0	0	13	0.00	1	49	2.04
10/3	1	17	5.88	1	18	5.6	0	13	0.00	2	48	4.17
10/10	1	16	6.25	0	17	0.0	0	13	0.00	1	46	2.17
10/31	0	15	0.00	0	17	0.0	0	13	0.00	0	45	0.00
Total	22	38	57.89	23	40	57.5	26	39	66.67	71	117	60.68
Average			2.92			3			3.49			3.11

abundance. A total of 12 feeding stations (hereafter systems) were used, with 6 each on the east and south transects and none on the north. The systems allowed quail to access a centrally located gravity driven feeder and were enclosed to prevent predation. The systems were spaced throughout their respective transects at approximately 1.6 km intervals and were baited with milo (*Sorghum bicolor*) prior to the treatment period. In the weeks before treatment, the milo was replaced with an untreated feed mix in order to acclimate the quail to the new food source. During treatment, a comparable feed mix with Fenbendazole was administered on the east transect. The north transect was left untreated and no feed was provided as a control measure, while the south continued to be provided with the untreated feed mix to account for the effects additional feed may have had on quail abundance.

2.4. Trapping and deployment of collars

Northern bobwhite quail were captured during two trapping periods from March–July 2015 and March–April 2016. The quail were live-trapped using a total of 62 double funnel walk-in traps (91.4X60.9 × 20.3 cm) placed at approximately 250–800m intervals along lightly traveled ranch roads. The traps were distributed amongst all three transects with 20 traps each on the north and east and 22 on the south. Trap locations were entered into the GPS and traps were baited with milo for two weeks before they were set. Traps were deployed next to cover and were sheltered with surrounding vegetation. Traps were checked and rebaited daily approximately 2 h after sunrise and 1 h before sunset.

Immediately following capture, the quail were placed in a cotton cloth bag and weighed using a digital hanging scale to ensure they were sufficiently large to accommodate the radio collar. Individuals were then assigned an aluminum leg band with a unique identification number. The birds were sexed based on the coloration of the plumage of the face and throat, and their age was determined by the presence or absence of buffed tips on the primary wing coverts. The presence of buffed tips indicated a juvenile, while the lack thereof was characteristic of an adult (Leopold 1945; Wallmo 1956). A cloacal swab was taken from each individual, and their condition score was recorded (Fig. 3). Northern bobwhites weighing at least 135g were fitted with a very high frequency (VHF) collar mounted radio-transmitter (Model A WE-Q; American Wildlife Enterprises, Monticello, FL, USA).

2.5. Radio telemetry

Collared birds were monitored for dispersion and survival using a handheld directional antenna (Wildlife Materials, Inc., Murphysboro, IL, USA), vehicle mounted antenna (RA-9, Magnetic mount antenna, Communications specialists, Inc, Orange, CA, USA) and handheld VHF receiver (R-1000 Telemetry receiver, Communications specialists, Inc, Orange, CA, USA). We conducted 19 telemetry sessions between 04/17/2015 and 10/16/2015 and 29 telemetry sessions between 03/21/2016 and October 10, 2016. The frequency of the signal determined the active or inactive status of each individual with an inactive signal indicating mortality. Transmitters emitted an intermittent pulse at >1 s intervals when active and switched to a <1 s interval when inactive. The transition from activity to inactivity occurred after the transmitter remained stationary for a period of 14–16 h.

To gauge reproductive success, collared hens were monitored extensively during April–August of 2015 and 2016. Using telemetry techniques, hens were located and checked for movement on a regular basis. If a hen was detected in the same location on at least two consecutive occasions, the operator would carefully approach and establish a perimeter around the nest. This was done by acquiring a signal with the antenna removed and circumnavigating the area, thus allowing the operator to narrow down the potential area without getting too close and causing nest abandonment. Next, a GPS point and notes of the location were documented so that the nest could be revisited while

Table 8
Results of our reproductive success monitoring during 2015 and 2016 field seasons. *Some females may have had multiples nests, these were included in the total number of nests recorded but were discounted when determining the percentage of females nesting.

		North	East	South	Total
2015	Total number of females monitored	18	9	12	39
	Total number of nests recorded* (% females nesting*)	7 (39%)	3 (33%)	1 (8%)	11 (28%)
	Total number of nests confirmed successful (% nest success)	2 (29%)	1 (33%)	1 (100%)	4 (36%)
	Average clutch size	12	14	13	13
	Average number eggs hatched/confirmed successful nest (% hatched)	10 (83%)	13 (93%)	12 (92%)	12 (92%)
	Confirmed nest depredations (% nest depredation)	2 (29%)	0 (0%)	0 (0%)	2 (18%)
	Female mortalities during nesting period (Mortality rate of monitored females)	3 (17%)	2 (22%)	2 (17%)	7 (18%)
2016	Total number of females monitored	16	19	17	52
	Total number of nests recorded* (% females nesting*)	10 (50%)	13 (63%)	6 (35%)	29 (50%)
	Total number of nests confirmed successful (% nest success)	7 (70%)	8 (62%)	6 (100%)	21 (72%)
	Average clutch size	14	14	12	13
	Average number eggs hatched/confirmed successful nest (% hatched)	13 (93%)	12 (86%)	11 (92%)	12 (92%)
	Confirmed nest depredations (% nest depredation)	3 (30%)	2 (15%)	0 (0%)	5 (17%)
	Female mortalities during nesting period (Mortality rate of monitored females)	2 (13%)	7 (37%)	4 (24%)	13 (25%)

the hen was foraging. If the hen's radio signal indicated that she was not in the vicinity, the area would be visually inspected for a nest. Otherwise, the operator would abort and return at the next opportunity. This process was repeated until a nest was found, in which case a GPS point and detailed observations were recorded. Field observations included nest location, number of eggs, state of the eggs, and nest success. Discovered nests were revisited if the hen was not heard within the vicinity and observations were updated.

2.6. Call counts

Spring call counts were conducted during three consecutive days on June 3–5th, 2015 and June 6–8th, 2016. The vehicle-based survey provided an index of male breeding abundance and was conducted simultaneously by three independent operators. Listening stations were randomly selected and spaced at > 800m intervals to prevent overlap of birds heard (Rollins et al., 2005; Crosby and Elmore 2012), the standardized distribution of the stations also prevented biases in site selection. The operator was present at the first listening post 30 min before sunrise and would record the number of rooster calls and the number of males heard for 5 min after the first call was heard. Afterward, the operator would proceed to the next waypoint and repeat the process for the remaining posts.

Covey counts were conducted on October 16–18th in 2015 and October 15–17th 2016 and were used to provide an index of northern bobwhite population abundance going into the fall and winter seasons. These counts consisted of a three-day survey of covey calls that were followed by vehicle-based roadside covey counts. The survey was operated simultaneously on the three transects, involved three operators and, unlike spring call counts, utilized only one listening post each day (Hernández and Guthery 2012). These stations were selected from previous points used for the spring call counts, separated by a minimum of 1.6 km, and spaced equidistant from each other. The operator was present at the listening station 45 min before sunrise and recorded the number of individual coveys for 20 min after the first covey call was heard (Hernández and Guthery 2012). The operator would then drive the transect and count the number of birds in each covey that flushed to determine the average number of bobwhites in a covey. This was done immediately after the conclusion of the listening session.

2.7. Parasite abundance estimates

Pretreatment verification of infections on all three transects were

Table 10
Expected, observed, and standardized residuals from chi squared test of qPCR results from cloacal swabs and fecal samples of bobwhite collected in Mitchell County, Texas in spring of 2015 and 2016. Observed values are in parentheses.

Transect-Year	*A. pennula* Positive	*A. pennula* ND	Standardized Residuals
East-2015	2 (8)	16 (10)	±2.86
East-2016	7 (9)	14 (12)	±0.86
North-2015	14 (9)	9 (12)	±2.06
North-2016	11 (8)	9 (12)	±1.31
South-2015	8 (8)	11 (11)	±0.03
South-2016	9 (9)	12 (12)	±0.11

Fig. 4. VHF radio collars recovered from quail mortalities showing evidence of likely avian predation (Damaged collars appear on right, with an undamaged collar on left as reference).

Table 9
Results of qPCR analysis from swabs and fecal samples collected in Mitchell County, Texas. ND indicates non-detect.

Transect-Year	*O. petrowi* High	*O. petrowi* Moderate	*O. petrowi* Low	*O. petrowi* ND	*A. pennula* High	*A. pennula* Moderate	*A. pennula* Low	*A. pennula* ND
East-2015	0	2	0	16	1	0	0	17
East-2016	0	1	0	20	0	6	1	14
North-2015	1	0	0	22	2	12	0	9
North-2016	0	0	0	20	0	9	2	9
South-2015	0	2	0	17	0	8	0	11
South-2016	0	0	0	21	0	7	2	12

completed and presented in Dunham et al. (2016b). Immediate effects of treatment were reported in Henry et al. (in press). The presences of *A. pennula* was evaluated using cloacal swabs or fecal samples collected from bobwhite captured in the spring following treatment on all three transects. DNA from cloacal swabs was extracted using Qiagen Fast Stool mini kits following Kistler et al. (2016). A quantitative polymerase chain reaction (qPCR) was then used to estimate eyeworm and cecal worm infection following Kalyanasundaram et al. (2018) with modifications in Leach et al. (in review). Infection intensity was then assigned according to Blanchard et al. (2019).

2.8. Statistical analysis

Referencing data from covey call counts, the mean number of calls per station were calculated for the north, east, and south transects for 2015 and 2016. This was done to account for the differences in the number of listening stations used across the three transects. In the case of spring call counts, the average daily rooster calls per transect were calculated. As aforementioned, both counts were conducted in a manner that assured a random sample with an independence of observations. Additionally, the dataset followed a normal distribution with equal variance thus satisfying the conditions for two-way analysis of variance (ANOVA). Using the independent variables of transect and year, we performed two-way ANOVA to test for statistical differences within the dataset. Significance was determined at $P \leq 0.05$ and a Tukey test was performed to determine significant differences between years and transects. A row by column (RxC) chi squared analysis was performed to determine differences parasite abundance between each transect and years. Individual bobwhite were grouped by positive or undetected to ensure assumption of a chi square analysis would be met.

3. Results

3.1. Trapping and telemetry

Trapping in 2015 and 2016 accounted for a total of 386 captures of bobwhite quail. During 2015, a total of 184 northern bobwhite captures were processed, 52 of these were recaptures. In 2016, trapping accounted for 202 bobwhite captures, 77 of which were recaptures (Table 5). A total of 111 and 117 bobwhites were fitted with VHF radio collars in 2015 and 2016, respectively. During 2015, we monitored an average of 72 birds per session and recorded a mortality rate of approximately 3 birds per session. In 2016, we tracked an average of 76 birds per session, during this period we noted a mortality rate of approximately 3 birds per session (Table 6). However, weekly mortality rates were highly variable within and between transects and there did not appear to be an overall difference between transects (Table 7). Throughout the course of our telemetry, we did not observe any evidence of birds moving between transects.

A total of 91 collared bobwhite hens were monitored for nesting activity in 2015 and 2016. During 2015, we monitored 39 females and located 11 nests, of which 36% were confirmed successful. We recorded an average clutch size of 13 with a 92% hatch rate. During 2016, we increased the number of females monitored to 52 and found 29 active nests of which 72% were successful. The average clutch size for 2016 was 13 with an observed hatch rate of 92% (Table 8). Additionally, three of the females monitored during 2016 attempted multiple broods although only one of these was confirmed successful. Several instances of nest depredation and female mortalities were also documented during the course of both nesting seasons. In 2015, 2 of the 11 nests monitored were confirmed depredated and 7 females were lost during female telemetry. In 2016, we observed depredations of 5 of the 29 nests monitored and recorded 13 hen mortalities (Table 8).

3.2. Call counts and bobwhite abundance

Covey call counts took place in mid-October of 2015 and 2016. During the 2015 survey, 4, 5, and 8 mean covey calls per station were heard on the north, east, and south transects respectively. In 2016, we heard 6, 16, and 6 mean covey calls per station across the north, east, and south transects, respectively (Table 1). Analysis of covey call counts indicated a significant effect of year and transect on the mean number of covey calls ($F_{2,12} = 11.83$, $P = 0.0015$). Tukey analysis revealed that mean covey calls on the east transect in 2016 were significantly higher than all other transects, while the remaining transects were statistically similar across all sampling periods (Table 2).

Spring call counts were conducted during early June of 2015 and 2016. The mean number of rooster calls heard daily in 2015 was 197, 445, and 234 on the north, east, and south transect respectively. In 2016, we recorded 506, 1257, and 634 average daily rooster calls on the north, east, and south transects (Table 3). Analysis of mean daily rooster calls revealed a significant interaction of year and location on spring call counts ($F_{2,12} = 5.98$, $P = 0.0158$). Tukey analysis confirmed a higher number of mean rooster calls on the east transect in 2016 when compared to any other sampling period or location (Table 4). There was no significant difference in rooster calls between the north and south in 2016, although the north in 2016 was significantly higher than all transects in 2015. Mean rooster calls on the south transect in 2016 were not significantly different from the east transect in 2015, and average rooster calls across all transects were statistically similar in 2015.

3.3. Helminth abundance

A total of 122 cloacal swabs and fecal samples were analyzed for helminth presence. 60 samples were collected in 2015 and included 18 from the east transect, 23 from the north transect, and 19 from the south transect. The remaining 62 were collected in 2016 with 21 from the east transect, 20 from the north transect, and 21 from the south transect. Results from qPCR analysis are shown in Table 9. An insufficient number of samples tested positive for O. *petrowi* so the analysis was only done for A. *pennula*. Chi squared analysis was significant ($\chi2(df = 5, N = 122) = 12.468$, $p = 0.029$). Examination of standardized residuals showed that the major contributors to the chi squared value were the East transect having fewer and the North greater than expected bobwhite test positive for A. *pennula* in 2015. However, that pattern repeated in 2016, but the discrepancy between expected and observed values was less substantial. Observed and expected values with standardized residuals are shown in Table 10.

4. Discussion

While habitat loss and dynamic environmental conditions have long been cited as major factors contributing to bobwhite decline, our research indicates that parasitic infections may have a more pronounced effect than was previously suspected. Our abundance surveys suggest that *O. petrowi* and *A. pennula* may drive bobwhite populations in our study area in Mitchell County, Texas. With the prevalence of these parasites confirmed through previous studies by Dunham et al. (2014b, 2016a), the east transect in our study area was treated with the anthelmintic Fenbendazole over the course of 2014–2015. Using spring call and fall covey call counts as an index of bobwhite numbers, we noted a significant increase in bobwhites on the east transect in 2016 with covey call counts indicating fall bobwhite populations approximately 300% larger than those of untreated areas. Furthermore, results from cooccurring research on the same site confirmed the efficacy of treatment at reducing *A. pennula* burden in bobwhite on the treated transect in 2015 and 2016 (Henry et al., in press). The results of the analysis in this study indicate that the immediate effects of fenbendazole treatment carry over into the next nesting season. This research taken together with the research presented in Henry et al. (in press) does indicate that reinfection is inevitable and at least once annual treatment is necessary to sufficiently suppress parasite infections in a population.

Although bird dispersal, habitat variability, and environmental conditions had the potential of affecting our observed bobwhite abundance, we attempted to control for these variables to isolate the effects of treatment on monitored bobwhite populations. During 2015 and 2016, we found no instances of movement between transects, and most of our bobwhites remained within 1 km of their capture location and was thus consistent with research on bobwhite mobility (Lehmann, 1946). The proximity between transects assured that environmental conditions were relatively uniform throughout the study area. Climate and habitat quality are known to affect bobwhite abundance, and all transects were within 10 km of each other to reduce the variability of these factors. Considering such equivalent environmental conditions, as well as the lack of dispersion between transects, the observed increase of bobwhite on the east transect in 2016 was likely a direct result of the treatment.

Although the consequences of *O. petrowi* and *A. pennula* infections in bobwhite are not yet fully understood, these parasites may influence bobwhite populations by decreasing breeding potential and individual survivorship. Reductions in reproductive success have been demonstrated to reduce abundance, and population dynamics of avian species with high reproductive rates, such as bobwhite and red grouse, are more likely to be influenced by reproductive success (Sæther and Bakke 2000; Stahl and Oli 2006). The decrease in fecundity caused by *T. tenuis* infection contributed significantly to red grouse population declines (Hudson 1986; Hudson et al., 1998), and it is conceivable that *O. petrowi* and *A. pennula* may likewise reduce the breeding capacity of bobwhite. In red grouse, *T. tenuis* infections in conjunction with malnutrition were a likely cause of reduced breeding production (Hudson 1986). Like grouse, bobwhite must fulfill key nutritional requirements in order to ensure successful reproduction, and parasitic infestations may compromise their ability to do so (Lehmann 1953). The reproduction metrics measured during this study were, however, quite similar between transects. This may be due to only providing treatment once a year in the later portions of the bobwhite nesting season. It is likely that providing treatment prior to the onset of the nesting season will be necessary to determine the effects of parasites on bobwhite reproduction.

Bobwhite require proper nourishment in order to survive periods of adversity, and parasites may also reduce their ability to cope with such conditions. However, no difference was found during 2016 covey call counts between the transect receiving unmedicated supplemental feed and the control transect. Thus, the additional nutrients in the base feed were not sufficient to improve bobwhite abundance on that transect. Although it is unlikely that parasitic infections kill bobwhite outright, it is plausible that they may increase the susceptibility of individuals to attrition during times of duress, thereby impacting populations. This potential of parasites to hinder the survivability of adult bobwhite is of particular concern, as survival of adults was found to have the greatest impact on population variance (Sandercock et al., 2008).

Bobwhite in the Rolling Plains ecoregion are subject to a wide array of stressors (Rollins 2007), and any potential weaknesses may decrease their ability to survive. In the course of our fieldwork in Mitchell County, we observed several periods of elevated mortalities that highlight some of the challenges affecting bobwhite survival. While mortality rates during regular telemetry in 2016 averaged 3.11 % (Table 8), there were several instances when these rates doubled. The first instance of increased mortalities coincided with the breeding season in late May and resulted in a peak weekly mortality rate of 7.78%. Females are vulnerable while incubating nests, and 13 of our 52 hens were lost in 2016 during the breeding period documented during this study. Another instance of increased attrition occurred late in June when a heat wave rapidly increased temperatures and ample time had passed for reinfections to occur at the study ranch. During this time, we measured several instances of temperatures in the vicinity of 40 °C. These temperatures approached the upper tolerance (41 °C) of adult female bobwhite (Case and Robel 1974) and are likely to have had an impact on mortalities. The final period of increased attrition occurred in late August and coincided with the migration of avian predators through the region (Allan and Sime 1943). Avian predators such as Cooper's Hawk (*Accipiter cooperii*) are confirmed bobwhite predators (Errington 1934), and multiple sightings of *A. cooperii* and other raptors were observed throughout the study ranch within this time period. Additionally, several collars recovered during this period possessed shear damage apparently caused by the beaks of avian predators (Fig. 4).

While it is unknown whether these increases in attrition were influenced by parasite load, our field observations illustrate how the pathological consequences of parasite infection may exacerbate periods of already high stress. Dunham et al. (2016a) found only ≤4% of bobwhite possessing an extreme infection of ≥40 eyeworms, suggesting that heavily infected individuals are highly susceptible to attrition. This study also found that the purported means of transmission and prevalence of these parasites were concomitant with periods of high rainfall, and it was suspected that large portions of bobwhite populations could quickly become infected during these times. Such epizootics could increase the vulnerability of quail populations to subsequent droughts and other adverse conditions, potentially leading to substantial mortalities. Considering the documented pathological consequences of these parasites, the potential for rapid infection of large numbers of individuals, and the diverse pressures affecting the survival of wild bobwhite, it is plausible that parasites play a key role in the survivability of quail in the Rolling Plains. Additionally, given the high prevalence of *O. petrowi* and *A. pennula* in the region, the potential effects these parasites have on affected quail populations must be taken into consideration.

5. Conclusions

Further research into how *O. petrowi* and *A. pennula* affect the fitness of individual bobwhite is necessary to better understand the consequences of infection on survivorship and reproduction. An understanding of these implications may yield additional methods of gauging the overall effects these parasites have on bobwhite populations in the Rolling Plains. Furthermore, the continuation of treatment and monitoring of bobwhite populations in this ecoregion is critical to understanding how parasite infection affects bobwhite abundance in the long term and under various environmental conditions. It is also advisable to increase the frequency of treatment to twice annually, once in late summer or early fall and once in early spring to help improve reproductive outcomes. Such an approach may also aid in determining the efficacy of anthelminthic treatment as a long-term mitigation tool for bobwhite declines. Considering the value of northern bobwhite as a game bird in the Rolling Plains, many landowners commit substantial resources to maintain sustainable populations of bobwhite on their properties. Innovative land management strategies, such as parasite control, have the potential of offering landowners a more economical way of maintaining their bobwhite, while also helping to offset bobwhite declines.

CRediT authorship contribution statement

Cassandra Henry: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Matthew Z. Brym:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Jeremiah Leach:** Writing – review & editing, Data curation, Methodology, Formal analysis. **Ronald J. Kendall:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors report no conflicts of interest.

Acknowledgements

We thank Park Cities Quail Coalition and the Rolling Plains Quail Research Foundation for the funding and support necessary to make this project possible. We also thank S. White, R. Bullard, and all those at Renderbrook Spade Ranch for their generous hospitality, assistance, and for allowing us ranch access. Finally, thank you to all the Wildlife Toxicology Lab members for their help with both field and lab work.

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