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HELMINTHS IN RUFFED GROUSE AT THE HOST'S SOUTHEASTERN RANGE BOUNDARY

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ABSTRACT: Carcasses of 268 ruffed grouse (*Bonasa umbellus*) collected in eastern Tennessee (USA) from October 1983 through February 1988 were examined between 16 January 1985 and 25 April 1988 for non-filarioid helminths. Three nematode species and one cestode species were found. The two most common parasites were the cecal worm *Heterakis bonasae* (81% prevalence, mean intensity ± SD of 62 ± 114) and the tapeworm *Echinolepis carioca* (27% prevalence, 30 ± 73 mean intensity). Age and sex of host were sufficient to predict infection by both common species. Condition of host and year and month of collection also had significant effects on prevalence. Intensity of cecal worm infection varied with age and condition of host and with region and month of collection.

Key words: Helminths, *Bonasa umbellus*, host density, parasite diversity, alternate hosts, host age, host sex, environment.

INTRODUCTION

The ruffed grouse (*Bonasa umbellus*) has a wide range in North America, from Alaska to Georgia (USA) (Johnsgard, 1983). The parasite fauna of this popular game bird has been studied extensively in the northern United States. The Great Lakes region approximates the ecological center of the ruffed grouse's range, where the species attains its maximum population density (Bump et al., 1947). Several surveys of the parasites of this bird have been published from the Great Lakes and from New England.

The only large survey of parasites in ruffed grouse of the southeastern United States was based on 200 grouse from West Virginia (USA) examined by Davidson et al. (1977). Their investigation included small samples from other southeastern states: six birds from northern Georgia and 11 from eastern Kentucky (USA). Our objectives were to determine the diversity, prevalence, and intensity of helminths in a large sample from the southeastern periphery of the host's range, represented by eastern Tennessee (USA), to compare parasite diversity to that in northern grouse, and to relate the parasite fauna to demographic and ecological factors in the host population.

Only three species of helminths were found in grouse from Georgia by Davidson et al. (1977). Though they found 14 species in West Virginia, the cecal worm *Heterakis bonasae* predominated numerically, with most other species being poorly represented. Spaulding (1958) found 12 species in 57 birds from Michigan (USA), while Erickson et al. (1949) found 11 species in 203 birds from Minnesota (USA). Checklists of ruffed grouse parasites from the Great Lakes region show totals of up to 53 species (Braun and Willers, 1967).

MATERIALS AND METHODS

Our sample of ruffed grouse was shot by cooperating hunters between 8 October 1983 to 28 February 1988. The four corners of the study area, clockwise from the northeastern corner, were approximately 36°36′N, 81°45′W; 35°00′N, 84°15′W; 35°00′N, 86°22′W; and 36°36′N, 85°15′W. Birds that could not be dissected within a few hours of death were frozen in sealed plastic bags by the hunter after removal of the breast, or treated by injection of the body cavity and gastrointestinal tract with buffered neutral formalin (BNF) followed by refrigeration at 4C. After these birds were dissected, their feces were collected from the

large intestine and preserved in BNF for later microscopic examination.

Dissections were performed to recover nonfilarioid helminths (Pritchard and Kruse, 1982). All organs evaluated were removed and placed in separate containers with buffered saline, and were inspected using a dissecting microscope with transmitted or reflected light.

The small and large intestines and cecum were sectioned longitudinally. After initial examination of the lumen, the inner wall was washed with a squeeze bottle and inspected again. It was then scraped with a blunt probe and washed again, the washings were screened through a 150-μm sieve, and the material remaining in the sieves was examined. When large volumes of material were encountered, the washings were allowed to settle. The overlying wash water was then decanted, and the settled material was poured into a glass pan on a light table and examined with a magnifying lamp.

The eyes were examined for nematodes under the nictitating membrane. The mouth was opened for inspection by disarticulating the jaws. The lumen of the trachea, esophagus, bile duct, and ureters was exposed by longitudinal section. The crop was slit so that it could be stretched while being viewed with transmitted light. The proventriculus was sectioned to examine the wall for cysts. The inner lining of the gizzard was removed to inspect for nematodes under it. The cloaca and bursa were everted for inspection. The lungs and liver were sectioned. The heart was dissected to examine the walls, chambers, valves, and major vessels. The renal calyces were exposed and inspected for trematodes.

The cecum is the site of heavy cecal worm infections in some southern galliform populations (Davidson et al., 1977; Dabney and Dimmick, 1977). This organ was inspected in its entirety in 116 grouse for which complete demographic information was available. The distal one-fourth of both branches, where most cecal worms were found, was examined in the remainder of the birds.

Nematodes were preserved in BNF, cestodes in alcohol-formalin-acetic acid (AFA). For identification of helminths, microscope slide-mounts were prepared according to procedures of Meyer and Olsen (1975). Nematodes were cleared and mounted with lactophenol. Cestodes were cleared with xylene, stained with Semichon's acetic carmine, de-stained with acid alcohol, dehydrated with ethanol, and mounted with Kleermount (Carolina Biological Supply, Burlington, North Carolina, USA).

The taxonomic keys of Cram (1927) and Schmidt (1986) were used to identify nematodes and cestodes, respectively. Representative specimens have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland) as United States National Museum Helminthological Collection Numbers 82008 to 82011.

Because fresh pre-dressed weights were not available for many of the carcasses, we developed an index of body condition for use in place of weight. Discrete fat deposits seen on the esophagus and trachea, crop, heart, liver, gizzard, intestinal mesenteries, and inner wall of the lower body cavity were rated visually on a relative scale of 0 to 5, with 0 corresponding to no visible fat and 5 to a very heavy deposit. The seven ratings were then summed to produce a condition index (CI) variable. Ratings for the gizzard were saved as a separate variable, for possible use as another indicator of body condition.

Weight of the fat adhering to the gizzard gives the best indication of condition in lieu of a whole body fat determination (Servello, 1985). Accordingly, gizzard fat weights were obtained for a subsample of birds. These weights were used to validate the condition index by correlation analysis.

The SAS procedures Catmod [categorical (log-linear) model] and GLM [general linear (analysis of variance) model] (SAS Institute Inc., 1985) were used to analyze prevalence and intensity of infection, respectively. Independent variables in the complete models were age, sex, and condition of host, as well as year, month, and region of collection. Condition variables were grouped into two levels in Catmod and into three in GLM. To further increase sample sizes in Catmod, gizzard rating was chosen over CI because more grouse had a gizzard rating than a complete CI. Analysis was limited to the most commonly encountered parasites, *Heterakis bonasae* and *Echinolepis carioca*. Associated tests (Chi-square contingency and goodness-of-fit, t test, Mann-Whitney Z) were performed using the Statgraphics package (Statistical Graphics Corporation, 1987).

Worm counts of cestodes were approximations, since in most cases they were collected as fragments. Analysis of intensity was limited to nematodes from those grouse in which the entire cecum was inspected. To reduce differences in variance among the subsamples and to normalize the data, worm burdens were transformed by taking the square root of the square root of the count.

Climatological data were taken from National Weather Service weather station reports from eastern Tennessee (National Oceanographic and Atmospheric Administration, 1983, 1985, 1986, 1987). The Erwin, Tazewell, and

TABLE 1. Sex (females : 1 male) and age (juveniles : 1 adult) ratios of ruffed grouse collected in eastern Tennessee, 1983 to 1988.

	Overall (n = 230)	Fall (n = 71)	January (n = 76)	February (n = 83)
Sex	0.81	0.92	0.93	0.73
Age	0.64	0.79	0.64[a]	0.56[b]

[a] Different from 1:1 ($P < 0.05$).
[b] Different from 1:1 ($P < 0.01$).

Allardt stations were chosen as geographic representatives of grouse distribution in the Blue Ridge Mountains, Ridge and Valley, and Cumberland Plateau physiographic regions, respectively. These regions occur east to west across the study area as listed.

RESULTS

Carcasses of 268 ruffed grouse were obtained; 229 (85%) of these birds were collected during the October 1983 to February 1984 hunting season. Remaining birds were pooled to create an adequate subsample for analysis. Most (n = 230) grouse were shot in January (33%) and February (36%), so grouse from the fall months (October through December) were pooled. The Ridge and Valley region accounted for 116 (63%) of the birds, but the other regions were not pooled because they were neither contiguous nor climatologically similar. Damage during collection or storage rendered 13 specimens unsuitable for examination.

Overall sex and age ratios of the sample were 0.81 females : 1 male and 0.64 juveniles : 1 adult (Table 1). The January and February age ratios were significantly ($P < 0.05$) different from a 1:1 ratio.

The nematodes *Heterakis bonasae* Cram 1927 (syn. *Heterakis isolonche* Madsen 1950), *Ascaridia bonasae* Wehr 1940, and *Cheilospirura spinosa* Cram 1927, along with the cestode *Echinolepis* (*Hymenolepis*) *carioca* (Ransom 1902) were recovered (Table 2). All species were found previously in ruffed grouse; only *A. bonasae* was not reported from other hosts as well. One grouse harbored four parasite species, three had three species, 66 (26%) had two species, and 145 (57%) had one species (weighted average of 1.3 species per infected host). Forty (16%) birds appeared free of parasites. Results of the fecal examination were inconclusive, with only one unidentified ascarid egg recovered. No differences were apparent between results obtained from carcasses that were fresh, frozen, or injected and refrigerated.

Condition index correlated well with gizzard fat weight (Pearson's r = 0.87, $P < 0.0001$, n = 17). Gizzard rating alone had a similar relationship (r = 0.87) to gizzard fat weight.

TABLE 2. Prevalence and intensity of helminths found in 255 ruffed grouse collected in eastern Tennessee, 1983 to 1988.

Species	Prevalence (%)	Intensity (worms/infected grouse)			
		Range	Mean ± SD	Median	Mode
Nematoda					
Ascarida, Heterakidae					
Heterakis bonasae (4)[a]	81	1–654	62 ± 114	13	5
Ascaridia bonasae (3)	2	1–3	—[b]	—	1
Spirurida, Acuariidae					
Cheilospirura spinosa (1)	1	1–27	—	—	—
Cestoda					
Cyclophyllidea, Hymenolepiidae					
Echinolepis carioca (2)	27	1–538	30 ± 73	6	1

[a] Numbers indicate location in host: 1, gizzard; 2, duodenum; 3, small intestine; 4, cecum.
[b] Sample size too small to calculate this value.

TABLE 3. Prevalence and intensity of *Heterakis bonasae* and prevalence of *Echinolepis carioca* infecting 255 ruffed grouse collected in eastern Tennessee from 1983 to 1988, by host age, sex, and condition.

	Age		Sex		Condition		
	Juvenile	Adult	Male	Female	Poor	Fair	Good
Prevalence[a]							
H. bonasae[b]	82	80	81	81	87	—[c]	73
E. carioca[d]	33°	24†	31°	22†	30°	—	29°
Intensity ($\bar{x}$)[e]							
H. bonasae	72° ± 123	59° ± 113	46° ± 106	84† ± 124	95° ± 161	70°† ± 116	32† ± 45

[a] Percent of subsample infected.
[b] No significant differences between values.
[c] No values for a mid-range class of condition.
[d] Values with different symbols (°,†) are significantly ($P < 0.05$) different (Chi-square).
[e] Mean worms per infected host ± SD; $n = 88$; means with different symbols are significantly ($P < 0.05$) different (Duncan's multiple range test).

Overall prevalences of the two helminths were independent of each other ($P = 0.13$).

For *H. bonasae*, the only reduced model that fit the prevalence data contained the effects of age, sex, and their interaction ($P = 1.0$). In all models none of the effects were significant ($P < 0.05$) individually (Tables 3 and 4).

Prevalence of *H. bonasae* was related to the interaction of sex and condition (two-way contingency $P = 0.01$), with males in poor condition having the highest prevalence (90%) and males in good condition the lowest (75%). Prevalence also was related to sex and age ($P = 0.06$), with juvenile males having the highest rate (87%) and juvenile females the lowest (75%). Juvenile males in poor condition had a prevalence of 92%, the highest of any age-sex-condition group. There were no significant ($P < 0.05$) differences from expectation based on proportion of the age, sex, or condition groups in the sample.

For *E. carioca*, the same reduced model comprising sex, age, and their interaction fit the prevalence data best ($P = 1.0$). A model composed of age, sex, condition, and the interaction effects of age-condition and sex-condition was also a statistically adequate predictor of prevalence, but it did not fit the data as well ($P = 0.12$) as the age-sex model.

In the complete model of *E. carioca* prevalence, age ($P = 0.05$), sex ($P = 0.04$), year ($P = 0.01$), and month ($P < 0.0001$) had significant effects (Tables 3 and 4). Prevalence in juveniles (33%) exceeded that in adults (24%), males (31%) exceeded females (22%), 1985 through 1988 (53%) surpassed 1983 through 1984 (25%), and January (36%) and February (45%) surpassed October through December (4%). There was no difference between January and February ($P = 0.4$). Prevalence was greater in 1985 through 1988 than in 1983–84 for all months of the hunting season ($P = 0.0005$).

The best-fitting model for intensity of *H. bonasae* infections comprised the factors age, sex, CI, month, year, and region, with interactions age-sex and month-year-region ($P = 0.000006$, $r^2 = 0.56$). Based on Duncan's multiple range test ($\alpha = 0.05$), females had more worms than males, grouse in poor condition had more than those in good condition, grouse had more worms in the winter months than in the fall, and grouse on the Cumberland Plateau had more worms than those in the Blue Ridge Mountains (Tables 3 and 4).

Based on a *t* test, adult males had lighter ($P < 0.01$) infections of *H. bonasae* (mean ± SD of 22 ± 38 worms) than the other age-sex groups combined (82 ± 131). Based on Mann-Whitney Z tests, adult

TABLE 4. Prevalence and intensity of *Heterakis bonasae* and prevalence of *Echinolepis carioca* infecting 255 ruffed grouse collected in eastern Tennessee from 1983 to 1988, by collection year, month, and region.

	Year		Month			Region[a]		
	1983 to 1984	1985 to 1988	Oct–Dec	Jan	Feb	CP	RV	BR
Prevalence[b]								
H. bonasae[c]	82	81	85	78	82	87	81	82
E. carioca[d]	25°	53†	4°	36†	45†	23°	31°	32°
Intensity[e]								
H. bonasae	48° ± 71	109° ± 189	31° ± 52	95† ± 179	74† ± 87	121° ± 203	53°† ± 75	13† ± 8

[a] CP = Cumberland Plateau; RV = Ridge and Valley; BR = Blue Ridge.
[b] Percent of subsample infected.
[c] No significant differences between values.
[d] Values with different symbols (°, †)are significantly ($P < 0.05$) different (Chi-square).
[e] Mean worms per infected host ± SD; $n = 88$; means with different symbols are significantly ($P < 0.05$) different (Duncan's multiple range test).

males had lighter ($P < 0.05$) infections than each of the other groups.

DISCUSSION

Low diversity of ruffed grouse parasites could be expected in eastern Tennessee, where the host population occurs at the edge of its species range. A species' density is usually lowest at the edge of its range (Krebs, 1978), with consequent reduced chances for transmission of parasites within that host population. The most likely alternate host of grouse parasites, the northern bobwhite quail (*Colinus virginianus*), also is generally uncommon in eastern Tennessee (R. W. Dimmick and P. I. Kalla, unpubl.). In addition, bobwhites typically occupy the flat, agricultural parts of that landscape, while grouse inhabit the forested slopes (R. W. Dimmick and P. I. Kalla, unpubl.). With the exception of the wild turkey (*Meleagris gallopavo*) as a known host of *E. carioca* (Maxfield et al., 1963), other galliform birds that might serve as alternate hosts are absent from the southeastern United States (Johnsgard, 1975). Lower parasite diversity away from the ecological center of the range of a widely distributed, non-migratory host also can be seen in the bobwhite, from which only three species of gastrointestinal helminths were reported in eastern Tennessee (Dabney, 1974). Kellogg and Prestwood (1968) observed that sparser bobwhite populations had fewer species of parasites.

Reports from ruffed grouse in northern states based on sample sizes comparable to ours, such as Levine and Goble (1947), have several times more parasite species than we found. Levine and Goble's (1947) 27 species from New York is the largest number in the literature, evidence that parasite diversity is greatest in that area. Maximum diversity could point to New York as the evolutionary center of the ruffed grouse's range, although overlapping distributions of alternate hosts could account for some of the parasites observed by Levine and Goble (1947).

Climate may account for the absence of trematodes in our sample. Trematodes of upland game birds generally require pulmonate snails as intermediate hosts (Olsen, 1974); these snails may occur at lower densities in the dry uplands of eastern Tennessee than elsewhere.

Davidson et al. (1977) found that *H. bonasae* was the predominant grouse parasite in West Virginia (USA), concurring with what we observed in Tennessee. This species is generally rare or absent in northern states (Erickson et al., 1949; Dorney, 1959; Davidson et al., 1977). The helminth fauna of Tennessee grouse bears less resemblance to grouse parasites elsewhere than to helminths of bobwhites in eastern Tennessee, from which Dabney (1974) collected *H. bonasae*, *C. spinosa*, and *Rhabdometra* spp.

Heterakis bonasae is common in southeastern bobwhites (Kellogg and Prestwood, 1968), a possible explanation for its high prevalence in Tennessee grouse. Where their ranges overlap, prevalence of this parasite is comparable in the two hosts (Venard, 1933; Dabney, 1974; Davidson et al., 1977; this study).

As prevalence and intensity of helminth infections differed among age-sex groups only during the fall months, the unequal sex ratios were inconsequential. Similarly, though the proportion of juveniles in the sample declined throughout the hunting season and juveniles had more common and heavier infections than adults, overall parasitism was lower in the fall; thus the effect of month was not confounded by age.

Age and sex together were sufficient to account for differences in prevalence of both common helminths. Because of dispersal and territorial competition (reviewed by Johnsgard, 1983), juvenile males may have been the most stressed age-sex group. Based on their CI, this group as a whole was in the poorest condition of any age-sex group. Mixed results on parasitism and condition of juvenile males have been obtained in other host species (McRae, 1978; Thomas, 1986; Moore et al., 1988).

The marginally higher prevalence of *E. carioca* observed in juveniles probably resulted from the insectivorous diet typical of that age group in summer. Other authors, such as Davidson et al. (1977), also have noted higher prevalence of parasitism in juveniles.

Annual mean temperature averaged over the period 1983 to 1984 was 0.7 degree C. less than in 1985 to 1988. Slightly cooler weather might account for the lower prevalence of *E. carioca* in 1983 to 1984, as insect intermediate hosts could have been less abundant then than in later years.

The reason for higher intensity of *H. bonasae* infection in females was unclear. Though there are many reports of juvenile hosts having more intense infections than adults (reviewed by Moore et al., 1987), we found none on sex influencing intensity in grouse or other southeastern galliforms.

The more sedentary habit of grouse in winter (Johnsgard, 1983) could have caused the higher intensities observed in January and February, by increasing hosts' contact with their own feces containing the infective eggs of *H. bonasae*.

Conditions during the collection period were wetter on the Cumberland Plateau than in the Blue Ridge Mountains. This difference could account for the higher intensity of *H. bonasae* seen on the Plateau, by making invertebrate transport hosts more available to the definitive host (Lund et al., 1966). Davidson et al. (1977) found a positive relationship between precipitation and intensity of *H. bonasae* infections in grouse of West Virginia, as did Prestwood (1968) for *Heterakis gallinarum* in wild turkeys of Mississippi (USA).

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