

BLOOD PARASITES IN SAGE-GROUSE FROM NEVADA AND OREGON

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ABSTRACT: Peripheral blood smears from 196 adult and yearling female greater sage-grouse (*Centrocercus urophasianus*) were examined for blood parasites (167 from the breeding and 29 from the brood-rearing season) to determine prevalence of blood parasites, to attempt to correlate infection with chick survival, and to establish base-line values of prevalence in sage-grouse from Nevada and Oregon (USA). Birds were captured and released on two study areas during 1999–2001; Sheldon National Wildlife Refuge (SNWR) in northwestern Nevada, and Hart Mountain National Antelope Refuge (HMNAR) in southeastern Oregon. Birds from a third study area, Beaty's Butte grazing allotment (BB) in southeastern Oregon, were sampled in 2000 and 2001. Overall, 19 birds (10%) were positive for *Leucocytozoon lovati* (= *L. bonasae*), 1 (0.5%) for *Plasmodium pedioecetii*, and 2 (1%) for microfilariae. Although prevalence of *L. lovati* on HMNAR was 39% during the breeding season in 1999 and 100% during the brood-rearing season in 2000, statistically, prevalence of *L. lovati* among study areas and years was not different. However, there were statistical differences between capture periods. Overall, 31% of the hens were positive for *L. lovati* during the brood-rearing season compared to 6% during the breeding season. There was no difference in packed cell volume between infected and non-infected birds and no difference between age-classes. However, mean sage-grouse productivity on HMNAR was higher (1.6 chicks/hen) for non-infected ($n=10$) compared to infected hens (0.7 chicks/hen; $n=7$), during 1999. Based on these limited observations on HMNAR in 1999, the possible effects that *L. lovati* may have on young sage-grouse could be detrimental to sage-grouse populations in Nevada and Oregon.

Key words: Blood parasites, *Centrocercus urophasianus*, *Leucocytozoon lovati*, Nevada, Oregon, *Plasmodium*, sage-grouse.

INTRODUCTION

Despite management and research efforts that date to the 1930s (Girard, 1937), breeding populations of sage-grouse (*Centrocercus urophasianus*), a once abundant sagebrush-steppe obligate, have declined by at least 17–47% throughout much of their range (Connelly and Braun, 1997). Current distribution extends south from southern Saskatchewan and Alberta (Canada), western North and South Dakota, Montana, Wyoming, Utah, Colorado, eastern Washington, Idaho, Oregon, Nevada, and northeastern California (USA) (Johnsgard, 1983; Drut, 1994). These declines have been attributed to degradation of sagebrush (*Artemisia* spp.) stands from excessive livestock grazing during the late 1800s and early 1900s, sagebrush control programs, agricultural conversions, introduction of exotic plant species, and alter-

ation of natural fire regimes (Dalke et al., 1963; Braun et al., 1977; Johnsgard, 1983; Crawford and Lutz, 1985; Martin, 1990; Drut, 1994). However, impacts of disease, including blood parasites, on sage-grouse populations have not been adequately investigated. To aid in this investigation, data is needed on the prevalence of blood parasites in sage-grouse in portions or all of their range.

Protozoan and helminth parasites of sage-grouse have been reported by workers in Colorado (Stabler et al., 1966; Stabler, 1974; Stabler et al., 1977), Wyoming (Boyce, 1990), California (Gibson, 1990), and Washington (Clark et al., 1968). Stabler et al. (1977) identified *Plasmodium pedioecetii*, *Haemoproteus canachites*, *L. bonasae* (= *L. lovati*), *Trypanosoma avium*, and microfilariae from blood films from 361 sage-grouse from Colorado.

The influence of hematozoan parasites,

including *L. lovati* (= *L. bonasae*), on the productivity of sage-grouse is unknown. This study was part of a larger study of sage-grouse ecology, and was conducted to investigate exposure to and prevalence of blood parasites in free-ranging sage-grouse, to determine if infection of hens with blood parasites is correlated to chick survival, and to establish base-line values of blood parasite prevalence in Nevada and Oregon. This is the first known study to evaluate the prevalence of blood parasites in sage-grouse in these states.

MATERIALS AND METHODS

Birds were sampled from three areas: Sheldon National Wildlife Refuge (SNWR; 41°45'N, 119°15'W) in northwestern Nevada; Hart Mountain National Antelope Refuge (HMNAR; 42°30'N, 119°40'W) in southeastern Oregon, and Beaty's Butte grazing allotment (BB; 42°05'N, 119°20'W), also in southeastern Oregon. Both SNWR and HMNAR are administered by the US Fish and Wildlife Service (USFWS), and BB is administered by the Bureau of Land Management (BLM). Two major land management practices in these study areas include livestock grazing and prescribed fire. On SNWR (232,294 ha), livestock grazing was eliminated in 1994; on HMNAR (87,253 ha) grazing was eliminated in 1990, and on BB (220,301 ha) livestock grazing has averaged 14,000 "animal unit months" since 1989 (BLM, unpubl. data). Prescribed fire is a primary rangeland management practice on all three study areas.

During their breeding (March–May) and brood-rearing (July–August) season, sage-grouse were located with spotlights and captured at night on their roosts with long-handled nets (Giesen et al., 1982; Wakkinen et al., 1992). Sex and age of captured birds were determined from wing molt and plumage characteristics (Crunken, 1963; Beck et al., 1975; Ottomeir and Crawford, 1996). Sage-grouse productivity (chicks/hen) was calculated as the number of chicks recruited to 1 August divided by the total number of radio-marked hens still alive at the onset of nesting.

Blood was taken from the cutaneous ulnar vein and fresh, non-anti-coagulated blood was used to make smears on glass slides. Smears were air-dried and sent unfixated to the Veterinary Diagnostic Laboratory (VDL), Oregon State University, Corvallis, Oregon for detection of blood parasites. Smears were stained in Wright's Giemsa and first scanned under low

dry magnification (100×) before being scanned under oil immersion (1,000×). Fifteen 100× fields with an average of 10,000 erythrocytes per field were scanned on each slide. Intensity of infection was recorded as "low" if a mean of one or fewer parasite was seen per 100X field, and "many" if there were more than a mean of one parasite per 100× field. Representative slides were sent to Dr. Ellis Greiner (Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA) for identification of species. Identification of species was based on morphology of gametocytes coupled to host specificity (Greiner, 1991).

In addition, packed cell volume (PCV) of 130 birds negative for *L. lovati* were compared to PCV of 15 infected birds. These samples were collected into Microtainer EDTA tubes, placed in refrigeration and kept cool for 8–36 hr until shipment overnight to VDL for analysis.

Comparisons of prevalence of *L. lovati* were made between capture periods (breeding and brood-rearing) and age-classes (adult and yearling), and among study areas (HMNAR, SNWR, BB) and years (1999, 2000, 2001) using data from female sage-grouse only. Only female sage-grouse were captured because this study was part of a larger study investigating reproductive parameters of hens. Comparisons were made with prevalence of *L. lovati* because only three birds were positive for other parasites. More breeding season capture sessions occurred at all study areas than brood-rearing capture sessions (Table 1), so tests for differences between capture periods were made using data from those years in which capture data from both seasons were available (2000 and 2001 for HMNAR and SNWR, and 2001 for BB).

Hen productivity (chicks/hen) between infected ($n=7$) and non-infected ($n=10$) was compared. However, statistical analysis was not conducted due to missing data. This comparison was made for hens on HMNAR in 1999 only, because insufficient data on hen productivity was available for SNWR and BB and for other years. All available data were used for tests between age-classes and among study areas. All analyses were performed in SAS (Statistical Analysis Systems, Version 8.0, Cary, North Carolina, USA) using Fisher's exact test for 2×2 tables (Freund and Wilson, 1997) and the Freeman-Halton test for larger tables (Freeman and Halton, 1951). The Freeman-Halton test is an extension of the Fisher's exact test for larger tables. A two sample *t*-test (Freund and Wilson, 1997) was used to test for differences in PCV between *L. lovati* infected and non-infected birds from both the breeding

TABLE 1. Prevalence of *Leucocytozoon lovati* in female sage-grouse (*Centrocercus urophasianus*) captured in the breeding (March–May) and brood-rearing seasons (July–August) during 1991–2001 at Sheldon National Wildlife Refuge, Nevada, and Hart Mountain National Antelope Refuge and Beaty's Butte Allotment, Oregon.

Study area ^b	Year	Prevalence ^a	
		Breeding season	Brood-rearing season
HMNAR	1999	39 (7/18)	
	2000	0 (0/23)	100 (5/5)
	2001	0 (0/19)	0 (0/8)
SNWR	1999	6 (1/16)	
	2000	5 (1/20)	0 (0/2)
	2001	0 (0/30)	33 (1/3)
BB	2000	8 (1/12)	
	2001	0 (0/29)	27 (3/11)

^a Prevalence is the percent of the sample infected with the parasite. The number in parentheses is the number positive for *L. lovati*/number sampled.

^b SNWR = Sheldon National Wildlife Refuge, Nevada; HMNAR = Hart Mountain National Antelope Refuge, Oregon, and BB = Beaty's Butte grazing allotment, Oregon.

and brood-rearing capture periods. All tests were 2-tailed because no *a priori* assumptions were made about the directionality of differences. Significance was $\alpha \leq 0.05$.

RESULTS

Peripheral blood smears from 196 female greater sage-grouse (103 adults, 93 yearlings) were examined for blood parasites during the sage-grouse breeding ($n=167$) and brood-rearing ($n=29$) seasons from two areas (HMNAR and SNWR) during 1999–2001, and in an additional third area (BB) in 2000 and 2001. Overall, 19 birds (10%) were positive for *L. lovati*, 1 (0.5%) for *Plasmodium pedioectii*, and 2 (1%) for microfilariae. In all cases, the intensity of infection was considered low. Prevalence of *L. lovati* and number of birds sampled by study area, year, and capture period can be found in Table 1.

Overall, 31% of the hens were positive for *L. lovati* during the breeding season compared to 6% during the brood-rearing season. Prevalence of *L. lovati* was different between capture periods for both HMNAR ($P \leq 0.001$) and BB ($P = 0.02$), but not for SNWR ($P = 0.18$). Therefore, tests for differences in prevalence among study areas were performed using data from the breeding and brood-rearing seasons sepa-

rately. No difference in *L. lovati* prevalence was detected between age-classes for any study area during either capture period ($P \geq 0.12$), so age-classes were combined for analyses.

Prevalence of infection with *L. lovati* on HMNAR during the 1999 breeding season was 39% and 100% during the brood-rearing season in 2000, which were higher compared to other years and study areas. However, statistically, there was no difference between HMNAR and SNWR in prevalence of *L. lovati* among birds captured during the breeding season during 1999–2001 ($P = 0.09$), nor among the three study areas during 2000 and 2001 ($P = 0.76$). Prevalence on HMNAR and SNWR during the 2000 and 2001 brood-rearing season also was not different ($P = 0.62$), nor was prevalence on the three study areas during 2001 ($P = 0.22$).

The mean PCV for birds infected with *L. lovati* captured during the breeding season was 51 ($SD \pm 5$, $n = 6$), compared to 53 ($SD \pm 8$, $n = 113$) for birds not infected, and these values were not different ($t = 0.75$, $df = 117$, $P = 0.45$). During the brood-rearing season, mean PCV of infected birds was 47 ($SD \pm 6$, $n = 9$) and mean PCV for birds not infected with *L. lovati* was 45 ($SD \pm 6$, $n = 17$), and there was no differ-

ence in these values ($t=-0.98$, $df=24$, $P=0.34$).

However, mean sage-grouse productivity on HMNAR was higher (1.6 chicks/hen) for non-infected ($n=10$) compared to infected hens (0.7 chicks/hen; $n=7$), during 1999. Based on these limited data, infection of hens with *L. lovati* may have reduced chick survival on HMNAR.

DISCUSSION

Several genera of protozoan parasites occur in the blood of birds. The most important are *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* species, but *Trypanosoma* species may also be found (Zinkl, 1986). The hematozoan parasites require two hosts, with schizogony (asexual replication) occurring in the bird and gametogony (sexual phase) and sporogony (asexual replication) occurring in the vector. Often these organisms can be present without evidence of disease. However, some of these organisms can cause disease, particularly when the host becomes debilitated or stressed. Furthermore, naive and aberrant hosts may be seriously affected by such organisms (Zinkl, 1986). Birds that are not the normal host for *Leucocytozoon* can succumb to a highly lethal disease known as aberrant leucocytozoonosis when infected. In this case, schizonts are numerous in tissues, but parasites are absent from the blood (Fraser, 1986).

The virulence of most *Leucocytozoon* spp. is unknown and all that is known about the majority is that they occur in certain hosts. Species which have been considered pathogenic include *L. simondi* in the anatids, *L. smithi* in domestic turkeys, and *L. caulleryi* in domestic chickens (Greiner, 1991). O'Roke (1934) first reported losses in anatids in Michigan due to *L. simondi* and Skidmore (1932) found disease in domestic turkeys infected with *L. smithi*. Reproductive potential of domestic turkeys was examined and significant decreases were noted in egg weight, percent egg production, and percent hatchability, but not in fertility in groups

of hens infected compared to hens not infected (Jones et al., 1972). Initially, other species of *Leucocytozoon* were considered to induce mortalities in such groups as grouse (Clarke, 1935), but newer research did not confirm the pathogenicity suggested earlier (Fallis and Hope, 1950).

Clarke (1935) indicated *L. lovati* (= *L. bonasae* Clarke) may have been responsible for mortality in ruffed grouse chicks (*Bonasa umbellus*). Erickson et al. (1949) and Erickson (1953) studied infections of *L. lovati* (= *L. bonasae*) during an 11 yr project and concluded that it was probably a harmless parasite that had little influence on the grouse cycle. They suggest that the parasite might cause juvenile mortality. However, the traditional view that blood parasites are only slightly or not pathogenic has been challenged, as recent research has revealed important alterations in infected birds (Bennett et al., 1993; Nordling et al., 1998; Raidal and Jaensch, 2000).

Sage-grouse in this study have a lower overall prevalence (11%) for blood parasites compared to that reported in most other states. Stabler et al. (1977) examined blood films from 361 sage-grouse from Colorado collected from 1974–76 and found 182 (50%) were positive for hemoparasites (*L. bonasae*, = *L. lovati*; 45% pos.), *H. canachites* (9%), *Trypanosoma avium* (8%), *Plasmodium pedioecetii* (1%), and microfilariae (3%). Gibson (1990) found only *Haemoproteus* spp. in 37.5% of 184 sage-grouse sampled over a 5 yr period in California. However, grouse in our study had such a low intensity of infection with blood parasites that we may have missed observing parasites in some birds, underestimating prevalence.

We found a higher prevalence of blood parasites (31%) during the July–August (brood-rearing) period compared to the March–May (breeding) period (6%). This has also been reported in other studies (Stabler et al., 1977; Barnard and Bair, 1986). Stabler et al. (1977) found the prevalence of blood parasites in sage-grouse in

Colorado increased from January–March (15%), to April (34%), to May (76%), and was 100% in birds sampled in July–September.

We found a declining prevalence of blood parasites in sage-grouse captured during the breeding season on HMNAR during 1999 compared to 2000 and 2001. The reasons for this are not clear but may be attributed to vector population abundance. Species of *Leucocytozoon* are transmitted by blood-sucking black flies (Simuliidae; Greiner, 1991). These vector populations may have been higher in 1999 due to the relatively high precipitation levels in the area in 1998, which caused higher water levels in the spring and early summer of 1999. Immature stages of black flies are restricted to running water (Fraser, 1986).

Packed cell volume can be used to detect anemia, which is often a clinical sign of blood parasitism in some avian species, including those infected with *Leucocytozoon* spp. (Amand, 1986; Fraser, 1986). Anemia can affect avian production (Fraser, 1986). Therefore, we used PCV in an attempt to detect anemia in infected birds. Amand (1986) reported PCV values between 37% and 53% for most avian species. Mean packed cell volume for infected birds we sampled was 51% ($SD \pm 5$), possibly indicating no anemia. We also found no significant differences in PCV between infected and non-infected grouse. This could suggest that infection with *L. lovati* was having no ill effects on the grouse resulting in anemia. However, chronic, low level, blood loss due to blood parasitism may not have resulted in significantly lower PCV values compared to acute blood loss (Amand, 1986). Also, the lack of low values of PCV could possibly be due to low intensity infections, which we observed in our birds. However, *L. lovati* may not be pathogenic in adult sage-grouse, even at higher intensities. Why there is a difference in chick survival between infected and non-infected hens is unknown. However, small sample size must be a consideration.

Based on our limited observations on HMNAR in 1999, the possible effects that blood parasites may be having on young sage-grouse in some years could be detrimental to sage-grouse populations in Nevada and Oregon. Research that will demonstrate the presence and effects of either the mature or immature stages of *L. lovati* in sage-grouse chicks will be necessary to determine its pathogenicity.

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