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GRAY WOLF DENSITY AND ITS ASSOCIATION WITH WEIGHTS AND HEMATOLOGY OF PUPS FROM 1970 TO 1988

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ABSTRACT: We examined weights and hematologic profiles of gray wolf (*Canis lupus*) pups and the associated wolf density in the east-central Superior National Forest of northeastern Minnesota (USA) during 1970 to 1988. We collected weight and hematologic data from 117 pups (57 females, 60 males) during 1 September to 22 November each year. The wolf density (wolves/800 km^2) trend was divided into three phases: high (72 ± 7), 1970 to 1975; medium (44 ± 2), 1976 to 1983; and low (27 ± 2), 1984 to 1988. Wolf numbers declined ($P = 0.0001$) 39 and 63% from 1970 to 1975 to 1976 to 1983 and from 1970 to 1975 to 1984 to 1988, respectively. Weight was similar between male and female pups and did not vary as wolf density changed. Mean hemoglobin ($P = 0.04$), red ($P = 0.0001$) and white blood cells ($P = 0.002$), mean corpuscular volume, mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin ($P = 0.0001$) did differ among the multi-annual phases of changing wolf density. Weight and hematologic data also were compared to values from captive wolf pups. The high, but declining wolf density was associated with macrocytic, normochromic anemia in wolf pups, whereas the lowest density coincided with a hypochromic anemia. Although hematologic values show promise for assessing wolf pup condition and wolf population status, they must be used cautiously until data are available from other populations.

Key words: *Canis lupus*, hematology, hemoglobin, wolf population density, wolf pups, wolf weights.

INTRODUCTION

Since 1973, the gray wolf (*Canis lupus*) has been protected in the 48 contiguous United States by the Endangered Species Act (U.S. Fish and Wildlife Service, 1978); however, in 1978, the wolf's status in Minnesota was downgraded to "threatened." Minnesota is the only one of the 48 states with a viable population of wolves, which has ranged from 1,200 in the mid-1970's to a present estimate of 1,550 to 1,750 (Fuller et al., 1991). Such numbers have afforded an opportunity for long-term research that has included investigations of numerous aspects of wolf ecology (Stenlund, 1955; Mech, 1975, 1986; Mech and Karns, 1977; Van Ballenberge and Mech, 1975; Fritts and Mech, 1981; Nelson and Mech 1981, 1986; Fuller, 1990; and others).

Findings of long-term research are critical to improving our understanding of wolf ecology, and they enhance our ability to manage wolves and their prey in areas with established populations. However, the success of recovery efforts for, and reintroductions of, this endangered species to its former range (U.S. Fish and Wildlife Service, 1987), also depends heavily upon knowledge generated and documented thus far.

For two decades, we have conducted research on wolf ecology in the central Superior National Forest (SNF), with our major focus being wolf-white-tailed deer (*Odocoileus virginianus*) relationships and trends in their respective populations (Mech, 1986; Nelson and Mech, 1986). As with all wildlife species, nutrition of wolves is linked to most aspects of their ecology (Robbins, 1983, p. 1). Beginning in the late 1960's, the deer population in our study area began a 10-yr decline (Mech and Karns, 1977), followed by a period of stability until the mid-1980's, and a subsequent increase through 1989 (Nelson and Mech, 1986; L. D. Mech, unpubl. data). The wolf population in the central SNF sharply declined from 1970 to 1975, was basically stable during 1976 to 1983, and subsequently decreased in 1984, with numbers remaining stable through 1988 (Mech, 1986; L. D. Mech, unpubl. data). Mech (1977a) suggested that the influence of nutrition on reproductive success of these wolves, including pup production and survival, was the primary mechanism whereby population change was effected.

Blood analyses may be used to assess the health of wolves, as well as of other wild and domestic species (Seal et al., 1975; Gates and Goering, 1976; Seal, 1978; Benjamin, 1981; Seal and Mech, 1983; Coles, 1986). Because pup condition may be a sensitive indicator of population changes (Mech, 1977b), our objective was to examine the association between wolf density in the central SNF from 1970 to 1988 and weights and hematology of wolf pups.

STUDY AREA

The study area encompasses approximately 2,060 km^2 in the east-central SNF (northeastern Minnesota, USA; 48°00'N, 92°00'W), and constitutes about 3% of Minnesota's total wolf range (Mech, 1986). The topography is undulant and includes swamps and rocky ridges. Elevation varies between 325 and 700 m (Mech, 1986). Conifer stands contain several species of pine (*Pinus* spp.) and spruce (*Picea* spp.), balsam fir (*Abies balsamea*), white cedar (*Thuja occidentalis*), tamarack (*Larix laricina*), trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). The area has a continental climate with ambient temperatures ranging from −35 C to 35 C (Anonymous, 1978; Mech, 1986). Snow depths of 50 to 75 cm occur between mid-November and mid-April. Although wolves consume deer, moose (*Alces alces*) and beaver (*Castor canadensis*), deer are the primary prey in our study area (Nelson and Mech, 1981).

MATERIALS AND METHODS

From 1 September to 22 November, 1970–88, 117 wolf pups (57 females, 60 males) were live-trapped with Newhouse No. 4 or 14 steel foot traps (Mech, 1974; Kuehn et al., 1986). From 1970 to 1979, pups were either immobilized physically or injected intramuscularly (i.m.) by pole syringe with a standard dose of 20 mg phencyclidine hydrochloride (HCl) (Sernylan, Bio-ceutics Laboratories, St. Joseph, Missouri 64502, USA), 100 mg ketamine HCl (Ketaset, Bristol Laboratories, Syracuse, New York 13201, USA), and 50 mg promazine HCl (Sparine, Wyeth Laboratories, Philadelphia, Pennsylvania 19101, USA). During 1980 to 1988, wolves usually received an initial i.m. injection of 100 to 400 mg ketamine HCl and 50 mg promazine HCl. When necessary for safe handling, we administered an additional 100 to 600 mg ketamine HCl.

Handling included weighing pups with a spring scale, drawing blood from a cephalic vein into serum tubes and vials coated with ethylenediamine tetraacetic acid, monitoring rectal temperatures, making morphological measurements, and ear-tagging each animal before release, as well as radio-collaring most of them. During 1976 to 1988, mean time between initial drug administration and blood collection was 29.4 ± 2.1 (n = 51) min. Because our handling protocol for wolves was the same throughout the 19 yr period, we are confident that the time between drug injection and blood sampling did not differ among multi-annual phases.

Blood samples were refrigerated until laboratory analyses which were conducted within five days. We assayed hemoglobin (Hb) by a spectrophotometric cyanmethemoglobin method; red and white blood cells (RBC and WBC) were counted in an automated cell counter (Coulter ZBI, Coulter Instruments Hialeah, Florida 33012, USA); and packed cell volume (PCV) was determined by a microhematocrit technique (Seal and Erickson, 1969; Seal and Mech, 1983). We calculated hematologic indices according to Benjamin (1981, pp. 128–129).

For comparison, we present weight and hematology data collected from seven captive, gray wolf pups (four females, three males) weighed and blood-sampled during early and late September, October, and November 1982. These pups were maintained on canine laboratory chow (Ralston Purina, St. Louis, Missouri 63164, USA) ad libitum, supplemented occasionally with road-killed white-tailed deer and a vitamin-mineral mixture. Pups were injected i.m. with a ketamine HCl (200 to 300 mg)-promazine HCl (50 mg) combination via pole syringe, and an additional 100–200 mg ketamine HCl if necessary for safe handling. Mean time between initial injection and blood collection was 21 ± 2 min. Handling procedure was similar to that of the wild pups.

The trend of annual wolf density from 1970 to 1988 was examined by simple linear regression. Mean wolf numbers in the study area dur-

TABLE 1. Weights and hematologic profiles of gray wolf pups during high (1970 to 1975), medium (1976 to 1983) and low (1984 to 1988) wolf density in the Superior National Forest, northeastern Minnesota, September to November 1970 to 1988.

	1970 to 1975			1976 to 1983			1984 to 1988		
	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n
Weight (kg)	18.0A[a]	0.9	43	19.6A	0.9	46	16.8A	1.1	25
Standardized weight (%)[b]	91.9A	3.6	43	102.4A	4.8	46	91.4A	6.4	25
Hematology[c]									
Hb (g/dl)	13.1A	0.2	45	13.5A	0.3	46	12.1B	0.4	26
RBC ($10^6/\mu$l)	4.5A	0.1	45	5.3B	0.1	46	5.5C	0.1	26
WBC ($10^3/\mu$l)	12.9A	0.6	45	15.6B	0.8	46	17.3B	0.7	26
PCV (%)	40.3A	0.6	45	42.8A	0.8	46	40.9A	0.9	26
MCV (fl)	94.1A	3.0	45	80.7B	0.7	46	74.0C	0.7	26
MCHC (g/dl)	32.5A	0.3	45	31.6A	0.3	46	29.5B	0.7	26
MCH (pg)	30.3A	0.8	45	25.5B	0.2	46	21.8C	0.6	26

[a] Mean values in a row with dissimilar letters are different ($P < 0.5$).

[b] Standardized weights were calculated as a percentage of weights of captive wolf pups of the same age (days) (Kuyt, 1972; Van Ballenberghe and Mech, 1975).

[c] Hb, hemoglobin; RBC, red blood cells; WBC, white blood cells; PCV, packed cell volume; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin.

ing the three multi-annual phases (1970–75, 1976–83, and 1984–88) (Mech, 1986; L. D. Mech, unpubl. data) were compared by one-way analysis of variance. To check weight and blood data for differences potentially attributable to stage of pup development, we compared data from the first and last third of the monthly data collection period (1 September to 22 November) by two-way analysis of variance (multi-annual phase versus portion of data collection period). Subsequently, weights of pups captured on different dates were standardized by calculating their percentage of weights of captive wolf pups of the same age (in days) (Kuyt, 1972) following the method of Van Ballenberghe and Mech (1975). The mean weight and values of hematological characteristics of each of our captive wolves were used to calculate descriptive statistics of the group. Weights of wild pups were tested by two-way analysis of variance (multi-annual phase versus sex) and hematologic characteristics by two-way analysis of covariance (covariate = weight). Multiple group comparisons were made by the method of least significant means.

RESULTS

Wolf density was negatively correlated ($r = -0.93$, $P = 0.0001$) with time from 1970 to 1988. Compared to the 1970–75 phase (72 ± 7 [SE] wolves/800 km^2), mean wolf numbers were 39 and 63% lower ($P = 0.0001$) during the 1976 to 1983 (44 ± 2 wolves/800 km^2) and 1984 to 1988 (27 ± 2 wolves/800 km^2) phases, respectively.

Although actual weights of male and female wolf pups were similar and did not significantly ($P = 0.14$) differ among the three multi-annual phases, mean weight tended to be less ($P = 0.06$) during 1984–1988 than 1976 to 1983 (Table 1). However, mean weight was 24.0% greater ($P = 0.02$) during the last third (20.7 ± 0.9 kg, $n = 27$) of the monthly data collection period (1 September to 22 November) compared with the first third (16.7 ± 1.0 kg, $n = 39$), reflecting growth during the collection period. Standardized weights did not vary among multi-annual phases (Table 1); however, during 1970–75, 1976–83, and 1984–88, 28, 23, and 48% of captured pups weighed less than 80% of captive pup standards, respectively. Standardized weights of females (101.0 ± 3.5%, $n = 58$) tended to be greater ($P = 0.11$) than for males (90.4 ± 4.3%, $n = 58$).

There were no temporal effects on hematologic characteristics during the monthly data collection period. Mean Hb ($P = 0.04$), RBC ($P = 0.0001$) and WBC ($P = 0.002$) counts, mean corpuscular volume (MCV), mean corpuscular hemoglo-

TABLE 2. Weight and hematologic profiles of captive gray wolf pups (four females, three males) in Minnesota blood-sampled six times during September to November 1982.

	$\bar{x}$	SE	n
Weight (kg)	20.3	1.4	7
Hematology[a]			
Hb (g/dl)	12.1	0.4	7
RBC ($10^6/\mu$l)	5.1	0.1	7
WBC ($10^3/\mu$l)	12.8	0.8	7
PCV (%)	36.1	0.9	7
MCV (fl)	71.0	1.1	7
MCHC (g/dl)	33.4	0.3	7
MCH (pg)	23.6	0.5	7

[a] Definitions as described in Table 1.

bin concentration (MCHC), and mean corpuscular hemoglobin (MCH) ($P = 0.0001$) differed among the phases of changing wolf densities (Table 1). Actual weight was a significant ($P = 0.0001$) covariate for Hb, RBC count, and PCV. Furthermore, Hb ($r = 0.38$), RBC counts ($r = 0.33$), and PCV ($r = 0.43$) were significantly ($P < 0.005$) correlated with standardized weights.

During 1970 to 1975, mean RBC and WBC counts were lowest and MCV and MCH were highest (Table 1). Additionally, during 1970 to 1975, mean Hb and MCHC were greater than values in 1984–1988, but similar to values of 1976 to 1983 (Table 1). During 1984 to 1988, Hb, MCV, MCHC, and MCH were lowest and RBC and WBC counts were highest (Table 1). Mean PCV did not vary with density, but was greater in females (42.4 ± 0.6%) than males (40.5 ± 0.6%). There was a significant ($P = 0.04$) interaction between phase and sex for Hb. Values in females increased from 1970 to 1975 (13.2 ± 0.3 g/dl, $n = 17$) to 1976 to 1983 (14.0 ± 0.4 g/dl, $n = 27$), then decreased during 1984 to 1988 (11.6 ± 0.6 g/dl, $n = 13$). Hemoglobin remained more stable in males (12.8 ± 0.2 g/dl, $n = 60$).

Hematologic values of captive wolves are presented in Table 2. Percentages of wild wolf pups during the three multiannual phases with values of selected hematologic characteristics statistically in-

TABLE 3. Percentage of wild gray wolf pups in the Superior National Forest, northeastern Minnesota (September to November 1970 to 1988) with hematologic values (indicative of anemia) more than two standard deviations (SD) below or above mean values of captive gray wolf pups (September to November 1982).

Hematological characteristics[a] Mean ± 2 SD	1970 to 1975	1976 to 1984	1984 to 1988
RBC ($10^6/\mu$l)			
(−) below 4.5	48.9	8.5	0.0
MCV (fl)			
(+) above 77	77.8	80.9	15.4
MCHC (g/dl)			
(−) below 32	26.7	55.3	69.2
MCH (pg)			
(−) below 21	2.2	0.0	19.2

[a] Definitions as described in Table 1.

dicative of anemia compared with our captive wolf pups are presented in Table 3.

DISCUSSION

The trend of actual and standardized weights of wolf pups did not reflect the deterioration of condition expected to be associated with the progressive decline in the wolf population in our study area from 1970 to 1988. However, proportions of wild pups weighing less than 80% of captive standards, indicated that their condition was poorest during 1970 to 1975 and 1984 to 1988 and somewhat improved during 1976 to 1983.

The highly significant variations of most hematologic characteristics from 1970 to 1988 reflected changes in the condition of pups. During 1970 to 1975, when wolf density was highest but declining precipitously, the diminished RBC counts and increased MCV's indicated a macrocytic, normochromic anemia (Benjamin, 1981, p. 130). Lower RBC counts, notably larger MCV's, and similar MCHC's compared with our captive pups (Tables 1, 2, and 3) supported this hematologic interpretation. Although there is no conclusive evidence of the specific cause of the anemia, deficiencies of niacin and/or folic acid may be implicated (Coles, 1986, pp. 39–40). Mean Hb (15.2 ± 1.5 g/dl), RBC counts

($6.2 \pm 0.6\ 10^6/\mu L$), and PCV ($49 \pm 4\%$) were greater and MCV (77 ± 3 fl) were smaller in 6-month old domestic dogs (*Canis familiaris*) than in our wild wolf pups (Brunden et al., 1970; Bulgin et al., 1970).

During 1970 to 1975, mean WBC count of wild pups (Table 1) was similar to values of our captive wolf pups (Table 2) and beagle pups ($11.3 \pm 3.0\ 10^3/\mu L$) of comparable age (Brunden et al., 1970; Bulgin et al., 1970).

During 1976 to 1983, when wolf density was moderate and relatively stable, increased RBC counts, similar to values in our captive pups, indicated an improvement in the condition of wild pups and appeared to agree with comparisons of standardized weights discussed earlier. Continued larger MCV's and lower MCHC's in a portion of our wild pups compared with our captive pups (Table 3) suggested a less optimal nutritional plane in the wild pups.

As wolf density continued to decline through 1984 to 1988, diminished MCHC and MCH values of the pups reflected a hypochromic anemia, possibly ascribable to iron, copper, or pyridoxine deficiencies (Benjamin, 1981, p. 133; Coles, 1986, p.39; Smith, 1989). Mean corpuscular volumes similar to those of captive pups suggested the normocytic character of the anemia. Certain prey organs (e.g., heart, liver) are high in iron and copper, whereas, copper and iron content in muscle mean is moderate (Smith, 1989). Messier (1987) also found lower Hb (12.6 ± 0.7 g/dl, $n = 8$) for wolf pups in an area of low prey density compared with one where the prey density was high (14.4 ± 0.5 g/dl, $n = 7$).

The mild to moderate leukocytosis in free-ranging pups from 1976 to 1988 compared with 1970 to 1975 eludes conclusive explanation. The nutritional compromise incurred by wolves may have made them more susceptible to infections. The degree of leukocytosis may be affected by infection intensity (Benjamin, 1981, p. 79). However, trauma and animal resistance during handling can have increasing effects on WBC counts as well (Benjamin 1981, p. 79). Direct comparisons with values of captive wolves would be questionable since the latter were not captured in steel foot traps.

It is difficult to hypothesize about the nutritional condition of wolf pups from three periods of different wolf density. During a high density, pups might be in good condition, reflecting the nutrition that supported the high density. Conversely, they may be in poor condition because of the high degree of competition. Similar conjecture could be made about the medium and low density phases of our wolf population history. Our wolf population remained low through 1988, and the hematologic profiles of the pups apparently reflected the greater nutritional stress that tends to more directly impact pups than adults when the prey base is low (Mech, 1977b).

In summary, it appears that hematologic values show promise for assessing the condition of 6- to 8-mo-old wolf pups and indicating the status of wild wolf populations. However, they must be interpreted cautiously until additional data have been obtained from other wolf populations, especially from those with increasing or stable numbers and adequate prey. Ultimately, however, they should prove useful for assessing the future health and condition of established wolf populations and wolves reintroduced to former range.

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