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HEMATOLOGIC VALUES OF THE ENDANGERED SAN JOAQUIN KIT FOX, *VULPES MACROTIS MUTICA*

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ABSTRACT: Between 1981 and 1982 blood samples were collected from 64 adult San Joaquin kit foxes, *Vulpes macrotis mutica*, in western Kern County, California. The goal of the study was to establish normal blood values for this endangered species, and to determine whether changes in them could be used to assess the possible effects of petroleum developments on these foxes. None of the values differed significantly between the sexes, or between foxes sampled in developed habitats compared with foxes sampled in undisturbed habitats. Mean values of Hb, MCH, MCHC, and WBC counts differed significantly between summer and winter. Average hematological characteristics were: RBC, 8.4 × $10^6/\mu l$; Hb, 14.5 g/dl (summer), 15.6 g/dl (winter); PCV, 46.9%; MCV, 56.3 fl; MCH, 17.8 pg (summer), 18.4 pg (winter); MCHC, 31.2 g/dl (summer), 33.2 g/dl (winter); and WBC, 6,200/μl (summer), 7,500/μl (winter). Comparisons of hematological data for kit foxes, coyotes (*Canis latrans*), and wolves (*Canis lupus*) confirmed a previously published observation that within mammalian families RBC counts are correlated inversely with body weight, and that MCV is correlated directly with body weight.

INTRODUCTION

The use of physiological indices as a tool in wildlife research and management has greatly increased in recent years. Physiological information on free-ranging animals may be useful in assessing health and condition of individual animals and of natural populations, and may indirectly serve as valuable indicators of habitat condition and changing habitat quality (Franzmann, 1972; Seal et al., 1975; Seal, 1977; Smith and Rongstad, 1980).

However, before hematology and serum chemistry studies can be used to assess the physiological state of a wild population, the range of normal blood values must be known (Lee et al., 1977). Only a limited number of hematologic studies of wild canids endemic to North America have been reported, including those of: the wolf (Dieterich, 1970; Hawkey, 1975; Seal et al., 1975; ISIS, 1982); coyote (Dieterich, 1970; Hawkey, 1975; Gates and Goering, 1976; Rich and Gates, 1979; Smith and Rongstad, 1980; ISIS, 1982); red fox, *Vulpes vulpes* (Kennedy, 1935; Spitzer et al., 1941; Dieterich, 1970; Hawkey, 1975; Brooks and Morris, 1979); gray fox, *Urocyon cinereoargenteus* (Schalm et al., 1975); and arctic fox, *Alopex lagopus* (Dieterich, 1970). The only published hematological data on the kit fox, *Vulpes macrotis*, was a single sample reported in the International Species Inventory System's (ISIS) Physiological Data Summary (ISIS, 1982).

The endangered San Joaquin kit fox is a small nocturnal subspecies endemic to arid regions of the San Joaquin Valley in south-central California. The Secretary of the Interior provided it with federal protection persuant to the Endangered Species Protection Act of 1966 (*Federal Register*, 32: 4001) because significant losses of habitat threatened the continued existence of the subspecies. It is presently protected under the Endangered Species Act of 1973 (Public Law 93-205). A detailed review of the life history of the San Joaquin kit fox is published in its Recovery Plan (O'Farrell, 1983).

Results of surveys conducted on much

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of the remaining public land within the range of the San Joaquin kit fox indicated that the greatest proportion of the extant population was located probably on the U.S. Department of Energy's (DOE) Naval Petroleum Reserve #1 (Elk Hills), Naval Petroleum Reserve #2 (Buena Vista), and adjacent portions of western Kern County (O'Farrell et al., 1980; O'Farrell and McCue, 1981). Hematological studies of San Joaquin kit fox were conducted to provide supplemental ecological information as part of a long-term applied research program on the possible effects of petroleum production on this endangered species. The overall goal of the research program was to ensure that attaining maximum petroleum production capability on Elk Hills would not jeopardize the endangered San Joaquin kit fox or its essential habitat.

The objectives of this study were to: 1) determine normal range of hematologic values for the San Joaquin kit fox; 2) assess health status of individual kit fox by identifying hematologic abnormalities; 3) provide baseline data for identifying metabolic, nutritional and/or toxic factors that may potentially influence hematologic values and thereby provide information on sources of morbidity and mortality; and 4) compare hematologic values of kit fox with those reported for other canids.

MATERIALS AND METHODS

Kit foxes were captured on the Elk Hills Naval Petroleum Reserve which is located approximately 48 km southwest of Bakersfield, Kern County, California. Elk Hills consists of a long narrow ridge system projecting eastward from the Temblor Range into the southwestern corner of the San Joaquin Valley. Elevation ranges between 88 m and 473 m above sea level.

Annual weather patterns consist of hot, dry summers and cool, damp winters. Lower Sonoran Grassland (Twisselmann, 1967) is the predominant vegetation association. It consists of a dense ground cover of red brome (*Bromus rubens*) and red-stemmed filaree (*Erodium cicutarium*), and an overstory of widely spaced shrubs including common saltbush (*Atriplex polycarpa*), spiny saltbush (*A. spinifera*), cheesebush (*Hymenoclea salsola*), matchweed (*Gutierrezia bracteata*), and bladderpod (*Isomeris arborea*). There are at least 23 species of mammals (O'Farrell, 1980).

Extensive petroleum field development is the primary activity on-site. The intensity of development varies between some areas devoid of well pads and associated disturbances, to others where the density of well pads exceeds one per 2 ha.

Animals were trapped in National® live-traps measuring 38 × 38 × 107 cm, that were baited with black-tailed jackrabbit (*Lepus californicus*) meat. Traps were set at dusk along trails or near den sites in both heavily developed and undisturbed habitats. They were checked at dawn to minimize the effects of heat-stress on captured animals.

Trapped foxes were coaxed gently into a cloth handling bag so that data and blood samples could be collected with a minimum of capture stress and excitement. No anesthetics were used. Data on sex, estimated age, weight, and standard body measurements were recorded for each animal.

Blood samples (6–9 ml) were taken from the jugular vein in 10 ml syringes and transferred immediately into sterile Vacutainers® containing ethylenediaminetetraacetic acid disodium salt (EDTA). Fresh blood smears were prepared on glass slides for differential counts of white blood cells.

After field collection all blood samples were refrigerated. They and the smears were shipped by mail to Veterinary Reference Laboratory, Inc. (P.O. Box 25978, Santa Ana, California 92799) on the same day they were collected.

The following hematologic values were measured as part of the Complete Blood Count (CBC) and differential (after Schalm et al., 1975): 1) erythrocyte or red blood cell count (RBC), measured with a Coulter Particle Counter, and expressed as the number of cells per microliter; 2) hemoglobin (Hb), measured with a spectrophotometer, and expressed as grams per deciliter; 3) packed cell volume (PCV), determined using a micro-hematocrit method, and expressed as the volume percentage of erythrocytes in whole blood; 4) mean corpuscular volume (MCV) expressed in femtoliters; 5) mean corpuscular hemoglobin concentration (MCHC) expressed in grams per deciliter; 6) mean corpuscular hemoglobin (MCH) expressed in picograms; 7) white blood cell count (WBC), determined with a Coulter Particle Counter, and expressed as number per microliter; and 8) white blood cell differential

count expressed as the proportion of 100 leukocytes observed on a blood smear that were neutrophils, lymphocytes, monocytes, eosinophils or basophils. Absolute numbers of each type were calculated by multiplying percent occurrence by the total WBC count.

Because of published discrepancies between leukocyte counts obtained using different techniques (Whittington and Comer, 1984) aliquots of four samples of kit fox blood were counted manually and with the Coulter Counter. The difference between the averages of the counts obtained manually (4,414 ± 738/μl, $\bar{x}$ ± SD) and with the Coulter Counter (4,400 ± 1,120/μl) was less than 1%.

Data sets for each parameter from adult foxes were examined using a Chi-square test for goodness of fit to determine whether they were distributed normally. The mean ($\bar{x}$), standard deviation (SD), and standard error (SE) were calculated for each data set. Aberrant values that differed from the mean by greater than three standard deviations were classified as "outliers" and excluded (Werner and Marsh, 1975; Lumsden and Mullen, 1978; Lumsden et al., 1979). Statistics for data sets were recalculated subsequently with the "outliers" removed. This statistical method was reported previously by Seal et al. (1975), Lee et al. (1977) and in the International Species Inventory System, Physiological Data Summary (ISIS, 1982). Ranges of normal values were calculated based on the mean ± two standard deviations, as traditionally reported in the veterinary medicine literature (Schalm et al., 1975). Data sets were compared for influences of sex, season, and habitat disturbance using Student's *t*-tests. Seasons were defined as summer (August–September) and winter (November–January).

RESULTS

Blood samples were collected from 64 (34 males, 30 females) adult (>6 mo old) kit foxes between 7 August 1981 and 7 January 1982. Tests of goodness of fit showed that data for all values were distributed normally. None differed significantly between the sexes; therefore the data were combined (Table 1). However, mean values for Hb, MCH, and MCHC differed significantly ($P < 0.05$) between summer and winter.

Average hematological characteristics were: RBC, 8.4 × 10^6/μl; Hb, 14.5 g/dl (summer), 15.6 g/dl (winter); PCV, 46.9%; MCV, 56.3 fl; MCH, 17.8 pg (summer), 18.4 pg (winter); MCHC, 31.2 g/dl (summer), 33.2 g/dl (winter). A complete list of the hematological measurements was published as an appendix in McCue and O'Farrell (1986).

Only four measurements related to characteristics of red blood cells and hemoglobin were excluded as "outliers": RBC (5.0 × 10^6/μl), Hb (8.1 g/dl, winter), and PCV (25.8%) for animal #1326; and MCV (69.0 fl) for #1784.

The average WBC count increased significantly ($P < 0.05$) between summer (6,200/μl) and winter (7,500/μl) (Table 1). Results of differential WBC counts obtained from 58 blood smears indicated that neutrophils and lymphocytes accounted for 80.4% and 15.5%, respectively, of the types of leukocytes observed (Table 1). An average of 2.7% of the leukocytes was classified as monocytes, and only 0.3% were eosinophils. No basophils were observed. Only the average number of eosinophils increased significantly ($P < 0.05$) between summer (5.2/μl) and winter (37.0/μl).

Ten measurements of white blood cells were determined to be "outliers" and were excluded from statistical analyses: animal #1328 had an elevated WBC count in winter (15,000/μl) due to neutrophilia (14,550 neutrophils/μl); #1654 had an excessive proportion (40%) of lymphocytes; #1348 (1,008 monocytes/μl, 14%) and #2066 (891 monocytes/μl) had monocytosis; and #1314 (840 eosinophils/μl, 14%) and #1672 (684 eosinophils/μl, 9%) had eosinophilia in winter.

None of the blood values differed between animals sampled in developed habitats compared with animals sampled in undeveloped habitats. Half of the foxes that had some hematologic values that were classified as "outliers" were sampled in undeveloped habitats and half were sampled in developed habitats.

TABLE 1. Hematologic values of adult San Joaquin kit foxes sampled in western Kern County, California between 1981 and 1982. Separate information is shown for those values that differed significantly between summer and winter.

Value	Sample size (n)	Range of observations	Mean ($\bar{x}$)	SD	SE	Normal range ($\bar{x} \pm 2$ SD)
RBC (no. × $10^6/\mu l$)	63	5.0–10.1	8.4	0.9	0.1	6.6–10.2
Hb (g/dl)						
Summer	30	11.1–17.4	14.5	1.5	0.3	11.5–17.5
Winter	28	8.1–18.3	15.6	1.5	0.3	12.6–18.6
PCV (%)	63	25.8–54.5	46.9	4.0	0.5	38.9–54.9
MCV (fl)	63	49.0–69.0	56.3	3.5	0.4	49.3–63.3
MCH (pg)						
Summer	30	14.6–20.1	17.8	1.1	0.2	15.6–20.0
Winter	29	16.3–19.7	18.4	0.7	0.1	17.0–19.8
MCHC (g/dl)						
Summer	30	28.2–35.5	31.2	1.9	0.3	27.4–35.0
Winter	29	30.8–37.2	33.2	1.6	0.3	30.0–36.4
WBC (no. × $10^3/\mu l$)						
Summer	30	1.0–9.2	6.2	2.1	0.4	2.0–10.4
Winter	28	4.3–15.0	7.5	1.8	0.3	3.9–11.1
Differential WBC counts						
Neutrophils (no. × $10^3/\mu l$)	57	2.0–14.6	5.7	1.6	0.2	2.5–8.9
(%)	58	58.0–97.0	80.4	8.4	1.1	63.6–97.2
Lymphocytes (no. × $10^3/\mu l$)	58	0.3–2.4	1.1	0.6	0.07	0.0–2.3
(%)	58	2.0–40.0	15.5	8.2	1.1	0.0–31.9
Monocytes (no. × $10^3/\mu l$)	56	0.0–1.0	0.2	0.1	0.02	0.0–0.4
(%)	56	0.0–14.0	2.7	1.7	0.2	0.0–6.1
Eosinophils (no./μl)						
Summer	23	0.0–89.0	5.2	17.9	3.7	0.0–41.0
Winter	27	0.0–840.0	37.0	62.8	12.1	0.0–162.6
(%)	56	0.0–14.0	0.3	0.6	0.1	0.0–1.5
Basophils (no./μl)	58	0.0				
(%)	58	0.0				

Band neutrophils were noted during five of 64 differential WBC counts and ranged between 1–7%.

DISCUSSION

No significant differences in hematologic values were noted between male and female kit foxes. Similar results were found for pen-raised coyotes (Rich and Gates, 1979), and captive wild coyotes (Gates and Goering, 1976). Differences between sexes in total WBC count for coyotes (Smith and Rongstad, 1980) and hemoglobin for wolf pups (Seal et al., 1975) have been reported. Hematologic reference values for male and female domestic dogs are similar and not typically differentiated (Schalm et al., 1975).

Hematologic data reported for domestic dogs (Schalm et al., 1975) differ from those of adult kit fox for the following values: average RBC ($\bar{x} = 6.8 \times 10^6/\mu l$) of dogs is less than that of kit foxes, while average MCV ($\bar{x} = 70.0$ fl), MCH ($\bar{x} = 22.8$ μl) and WBC ($\bar{x} = 11{,}500/\mu l$) of dogs is higher than values for kit foxes.

It is noteworthy that RBC ($8.0 \pm 1.0 \times 10^6/\mu l$) and MCV ($60.0 \pm 7.0$ fl) (ISIS, 1982) of the fennec, *Fennecus zerda*, a small fox (<1–1.5 kg) endemic to the Sahara Desert of North Africa, are quite similar to those of the kit fox, its North American ecological equivalent.

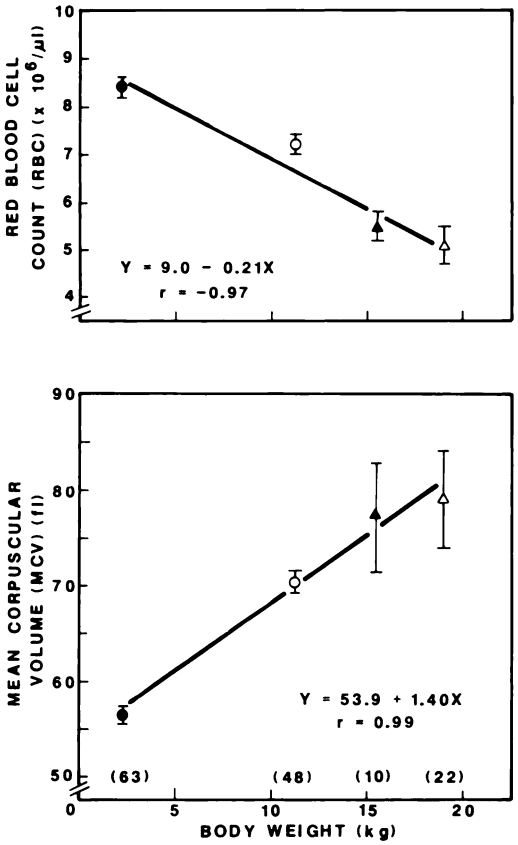

FIGURE 1. Relationship between red blood cell counts (RBC), mean corpuscular volume (MCV), and body weights for kit foxes (closed circles, average weight = 2.26 ± 0.32 kg), coyotes (open circles, average weight = 11.3 kg) (Rich and Gates, 1979), and wolves (females, open triangles, average weight = 15.5 ± 5.9 kg; males, closed triangles, average weight = 19.1 ± 7.1 kg) (Seal et al., 1975). Numbers in parentheses are sample sizes; means are bracketed by 0.95 confidence intervals.

The increases in Hb and MCHC values and WBC counts of kit foxes observed between summer and winter, and the lack of seasonal change in MCV were similar to seasonal hematological patterns reported for captive gray wolves (Seal and Mech, 1983). Unlike gray wolves, however, similar seasonal trends were not observed in RBC counts (8.5 ± 0.8 × $10^6/\mu l$, both seasons) and PCV values (47.3 ± 3.2%, summer; 46.8 ± 4.3%, winter) of kit foxes.

Because the decrease in MCHC during the summer was not accompanied by a change in red cell size (MCV) it could be interpreted as a normocytic and mildly hypochromic seasonal "anemia" as suggested by Seal and Mech (1983) for gray wolves. Similarly the white cell elevation in winter may reflect greater vulnerability to infections which was speculated for gray wolves also (Seal and Mech, 1983).

Five CBC values were identified as "outliers"; three of which were from female #1326. The hematologic profile, or hemogram, for this animal indicated the presence of a non-responsive, normocytic, normochromic anemia (MCV = 52 fl, MCHC = 31.3 g/dl). This suggests a chronic disease process or bone marrow abnormality (Schalm et al., 1975).

The other "outliers" included: an increased MCV (macrocytic), for #1784; and a high total WBC for #1328 that was characterized primarily by a neutrophilia (97% of total), and lymphopenia (300/μl, 2% of total). This altered leukocyte pattern is highly suggestive of a stress response to capture.

The hematologic changes that occur during the stress response have been documented for a variety of wildlife species (Franzmann, 1972; Gates and Goering, 1976; Karns and Crichton, 1978; Seal and Hoskinson, 1978; Smith and Rongstad, 1980). The classic leukocyte pattern seen with stress is a leukocytosis, consisting of a neutrophilia, monocytosis, lymphopenia and eosinopenia (Schalm, 1980).

Methods of capturing and handling kit foxes were developed to ensure the safety of both the animal and the researcher, and to minimize stress on captured animals. Inevitably some individuals became hyperexcited when first captured and during the initial stages of handling. No attempt was made to quantify amounts of stress observed in individual animals; instead, every effort was made to minimize or prevent it.

In addition to the high absolute neutrophil count for #1328, eight other differential WBC count measurements were identified as "outliers." A monocytosis was noted for #1348 and #2066 which may have been caused by a chronic disease process. However, when #1348 was recaptured 8 mo later the absolute monocyte count (492/μl) was within the normal range. The proportion of lymphocytes observed in #1654 was abnormally high, but the actual number of lymphocytes (1,720/μl) was well within the normal range.

Two foxes, #1314 and #1672, displayed an eosinophilia in winter, both in absolute number and percent. No other abnormalities were noted on the hemogram of either animal. The eosinophilias may have been induced by either parasitism, allergic reactions, or chronic disease processes.

The fox whose differential had 7% band neutrophils sustained a compound fracture of the mandible approximately 4½ mo prior to blood sample collection. A persistent infection initiated at the time of injury may have influenced the differential.

None of the blood values or "outliers" differed significantly between foxes sampled in developed and undeveloped habitats. Either there were no hematological changes caused by the effects of petroleum development activities on kit fox, or the changes were too subtle to be measured by the methods employed.

As in other field studies of free-ranging wildlife (Seal et al., 1975; Lee et al., 1977; Seal and Hoskinson, 1978; Smith and Rongstad, 1980), blood was not analyzed immediately after collection. Instead, it was refrigerated and mailed to the analytical laboratory that afternoon. There was a delay of 24 to 48 hr between the time of sample collection and analyses. Some of the characteristics of the blood samples may have been altered during this delay and attendant, uncontrolled temperature changes. However, this method of collecting and shipping blood for analysis is routinely used by veterinary practitioners, and the assumption was made that it had little to no influence on the results.

Several authors previously described relationships between hematologic values and body mass (Dunaway and Lewis, 1965; Hawkey, 1975; Schalm, 1980). Dunaway and Lewis (1965) reported that RBC counts were related inversely to body weight, and that MCV was related directly to body weight within several taxonomic families investigated. Because Hb and PCV values of mammalian blood are relatively constant for all species regardless of mass (Hawkey, 1975), changes in body weight within a taxonomic family are correlated with changes in RBC counts and MCV.

Data reported for wild canids follow the pattern described by Dunaway and Lewis (1965) and Hawkey (1975). Hemoglobin concentrations and PCV values are relatively constant, and there is a direct correlation between increased body weight and increased MCV ($r = 0.99$) and decreased RBC counts ($r = -0.97$) within three North American canids of varying masses (Fig. 1). A trend toward increased MCH with increased body weight of canids is also apparent, as would be predicted on the basis of a relatively constant hemoglobin concentration (Hb) and a decreased RBC.

In classic studies of energy metabolism, Kleiber (1961) reported an inverse relationship between body size and weight-specific metabolic rate, where smaller species have higher metabolic rates than larger species. This relationship was confirmed for canids, because the metabolic rate of kit foxes has been measured to be approximately twice that of coyotes on a weight-specific basis (Golightly and Ohmart, 1983).

The primary function of the erythro-

cyte is to carry hemoglobin (Schalm et al., 1975). Small erythrocytes (decreased MCV) allow a greater number (increased RBC) of erythrocytes per unit volume while maintaining a constant PCV. It is hypothesized that gas exchange is enhanced when hemoglobin is distributed in a greater number of smaller sized erythrocytes having an increased total surface area. It may, therefore, have been an adaptive advantage for kit foxes with a high weight-specific metabolic rate to have evolved a high RBC count and low MCV, while maintaining constant Hb and MCHC. This may allow an increased amount of oxygen transport and exchange, while PCV was maintained relatively constant, thereby avoiding problems associated with hemoconcentration and increased viscosity of blood.

Several hematologic values for San Joaquin kit foxes were quite distinct from values for other larger wild canids endemic to North America, or for domestic dogs. Therefore, normal ranges established for kit foxes should be used to monitor health status of individuals, and of the population on Elk Hills that is being affected by petroleum developments. This study also provided baseline hematologic data that will be useful during subsequent investigations of the possible metabolic effects that changes in such things as degraded environmental quality and nutritional status (i.e., prey base) may have on this endangered species.

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