

**BLOOD CHARACTERISTICS  
OF  
SAN JOAQUIN KIT FOX  
(Vulpes velox  
macrotis)  
AT CAMP ROBERTS  
ARMY NATIONAL GUARD  
TRAINING SITE,  
CALIFORNIA**

**September 1992**

**SANTA BARBARA OPERATIONS**

130 Robin Hill Road  
Goleta, California 93117

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By

**William G. Standley and Patrick M. McCue**

**September 1992**

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Work performed for the U. S. Departments of the Army and Air Force  
National Guard Bureau  
Aberdeen Proving Grounds, Maryland 21010-5420  
through an agreement with the U. S. Department of Energy,  
Nevada Field Office  
under contract No. DE-AC08-88NV10617

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## **ABSTRACT**

Hematology, serum chemistry, and prevalence of antibodies against selected pathogens in a San Joaquin kit fox population (Vulpes velox macrotis) were investigated at Camp Roberts Army National Guard Training Site, California, in 1989 and 1990. Samples from 18 (10 female, 8 male) adult kit foxes were used to establish normal hematology and serum chemistry values for this population. Average values were all within the normal ranges reported for kit foxes in other locations. Three hematology parameters had significant differences between male and female values; males had higher total white blood cell and neutrophil counts, and lower lymphocyte counts. There were no significant differences between serum chemistry values from male and female foxes. Prevalence of antibodies was determined from serum samples from 47 (26 female, 21 male) adult kit foxes and 8 (4 female, 4 male) juveniles. Antibodies were detected against five of the eight pathogens tested: canine parvovirus, Toxoplasma gondii, Leptospira interrogans, canine distemper virus, and canine hepatitis virus. Antibodies were not detected against Brucella canis, Coccidioides immitis, or Yersinia pestis.

## **ACKNOWLEDGEMENTS**

The assistance of the many people that collected data during this study is greatly appreciated. The following persons assisted with trapping and drawing blood from kit foxes: W. H. Berry, K. Charlton, C. G. Logan, E. A. Reese, and K. A. Spencer. J. Ando assisted with data storage. Dr. M. C. Anderson provided assistance with statistical analyses. Dr. J. Johannes provided access to laboratory equipment.

The cooperation and assistance of the following California Army National Guard personnel are gratefully acknowledged: Col. D. Baird, Col. W. Mongolo, Col. J. Scully, Maj. E. Martzen, Maj. A. Vargas, Lt. D. Lee, Sgt. J. Noble, Sgt. G. Perry, Sgt. K. Remo, Mr. A. Davis, Ms. A. DeBevec, Mr. B. Duke, and Ms. J. Eliason.

Permission to trap and handle San Joaquin kit foxes was granted by the U. S. Fish and Wildlife Service through permit PRT 683011 and a Memorandum of Understanding between the California Department of Fish and Game and EG&G Energy Measurements, Inc. (EG&G/EM).

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## 1. INTRODUCTION

The San Joaquin kit fox population (Vulpes velox macrotis, formerly Vulpes macrotis mutica; Dragoo et al. 1990) was listed as endangered by the U. S. Fish and Wildlife Service in 1967 (U. S. Department of the Interior 1967). The primary cause of the fox's endangered status is habitat loss to agricultural, industrial, and urban developments (O'Farrell 1983).

The physiological characteristics of blood samples taken from free-ranging animals provide a method of assessing the health and condition of individual animals, the population, and habitat quality (Seal et al. 1975). Physiology of blood from San Joaquin kit foxes inhabiting the Naval Petroleum Reserves in Kern County, California has been reported as part of an investigation of the effects of petroleum extraction activities on kit fox (McCue and O'Farrell 1987, 1988, 1992).

San Joaquin kit fox blood parameters were investigated as part of an investigation of the effects of military activities on kit foxes at the Camp Roberts Army National Guard Training Site (Berry et al. 1992). Objectives were as follows: 1) measure standard hematological and serum chemistry values; 2) define the normal range of hematology and serum chemistry values for kit foxes inhabiting Camp Roberts; and 3) determine the presence of antibodies against selected pathogens in individual foxes.

## **2. STUDY AREA**

Camp Roberts is a military training site operated by the California Army National Guard with funding from the National Guard Bureau. It is located approximately 43 km east of the Pacific Ocean, midway between Los Angeles and San Francisco along U. S. Highway 101 in San Luis Obispo and Monterey Counties (Figure 1). Camp Roberts encompasses 172 km<sup>2</sup> of mostly gently rolling hills that form a transition zone between the Salinas River floodplain and the steep foothills of the Santa Lucia Mountains. Elevations range between 161 and 521 m above sea level. Average annual rainfall is 28.5 cm of which over 90% occurs between November and April (Nakata and Associates 1987). Fog is common in winter months. Dominant vegetation associations are grassland, oak (Quercus spp.) woodland, mixed chaparral, and riparian habitat. Kit foxes occur mainly in the grasslands and low to medium density oak woodlands, although they also occupy developed areas of the base where they live under buildings (Reese et al. 1992).

### 3. METHODS

Blood samples were collected from kit foxes after they were captured in wire mesh live-traps following methods described in O'Farrell (1987). Blood samples (8-10 ml) were drawn from the foxes' jugular vein with a 12-ml syringe. Part of the sample was combined with ethylenediaminetetraacetic acid disodium salt (EDTA) for whole blood studies, and part was left free of additives for determination of serum chemistry and prevalence of antibodies. Blood smears were prepared on glass slides for white blood cell differential counts. Blood samples were refrigerated after field collection. A portion of each sample was centrifuged to obtain serum, which was shipped along with the whole blood and blood smears to an analytic laboratory. All blood parameters were analyzed by one of two reference laboratories: Veterinary Reference Laboratory (Anaheim, CA) and Veterinary Medical Teaching Hospital, Department of Immunology, University of California, Davis (Davis, CA). Two additional blood samples were collected from kit foxes found dead in fresh condition. These samples were only used to determine prevalence of antibodies.

Hematologic parameters measured were as follows: red blood cell (RBC) count; hemoglobin (HGB) content; packed cell volume (PCV); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); nucleated red blood cells (NRBC) expressed as the number per 100 white blood cells (WBC); band neutrophil percentage and absolute number; WBC count; and absolute number and percentage of five classes of leukocytes: neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Serum chemistry parameters analyzed were as follows: aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, blood urea nitrogen (BUN), creatinine, BUN:creatinine ratio, cholesterol, alkaline phosphatase, glucose, calcium, phosphorus, calcium:phosphorus ratio, total protein, albumin, globulin, albumin:globulin ratio, sodium, potassium, sodium:potassium ratio, chloride, carbon dioxide (CO<sub>2</sub>), creatine phosphokinase (CPK), gamma GT, uric acid and lactic dehydrogenase (LDH). The mean, standard deviation (SD) and standard error (SE) were calculated for each parameter. Aberrant values that differed from the mean by greater than three standard deviations were classified as outliers. Statistics were recalculated after the outliers were excluded. Lilliefors' Kolmogorov-Smirnov test was used to determine if the data were normally distributed. The student's t-test was used to determine significant differences between male and female mean blood values. Differences were considered significant whenever probabilities were  $\leq 0.05$ . Normal values were presented as the mean plus or minus two standard deviations, as traditionally reported in veterinary medicine literature (Lumsden and Mullen 1978).

Serological tests were performed to determine presence of antibodies against pathogens of the following diseases that are known to occur in kit fox or other canids, or known to have severe impacts on wildlife populations: brucellosis (Brucella canis), leptospirosis (Leptospira interrogans serotypes canicola, grippotyphosa, hardjo, icterohaemorrhagiae, and pomona), canine parvovirus, toxoplasmosis (Toxoplasma gondii), coccidioidomycosis (Coccidioides immitis), infectious canine hepatitis virus, sylvatic plague (Yersinia pestis), and canine distemper virus. If antibodies against canine parvovirus were detected, the serum was then treated with 2-mercaptoethanol and retested. A fourfold or greater decrease in the titer (the relative amount of antibodies in the serum) after treatment suggests the fox was exposed within

2 weeks of when the blood was drawn. The presence of antibodies against rabies virus was not determined because infected animals generally die before antibodies appear in their blood.

## 4. RESULTS

There were significant differences between blood characteristics determined by the two reference laboratories (EG&G/EM, unpublished). Consequently, only data from one laboratory (Veterinary Reference Laboratory Inc.) are presented for hematology and serum chemistry. The data presented were chosen because they provided the largest number of samples collected within a single season. These data were analyzed by the same laboratory used in the previous analysis of kit fox blood characteristics (McCue and O'Farrell 1987), making the results more directly comparable. All data were used to determine prevalence of antibodies.

### 4.1 HEMATOLOGY

Normal values and associated descriptive statistics for all hematological parameters for 18 adult (10 female, 8 male) San Joaquin kit fox blood samples are presented in Table 1. All samples analyzed were collected between September 7 and October 5, 1989. Complete hematology data for each fox are included in Appendix A. No values were excluded as outliers.

Some samples were too small to measure all parameters. The data for all parameters were normally distributed, except for WBC differentials. There were three parameters with significant differences between sexes; males had a higher WBC count ( $t = -2.66$ ,  $df = 16$ ,  $P = 0.017$ ) and neutrophil count ( $t = -3.08$ ,  $df = 16$ ,  $P = 0.007$ ), and females had a higher percentage of lymphocytes ( $t = 2.40$ ,  $df = 16$ ,  $P = 0.029$ ). No nucleated RBCs were observed on 17 blood smears, and band neutrophils were observed on 3 of 17 (17.6%) blood smears and ranged between 2 and 4% ( $92 - 428/\text{mm}^3$ ).

### 4.2 SERUM CHEMISTRY

Normal values and associated descriptive statistics for all serum chemistry parameters for 18 adult San Joaquin kit foxes are presented in Table 2. Complete serum chemistry data for each fox are included in Appendix B. One value was excluded as an outlier; female #4157 had an alkaline phosphatase concentration of 140 IU/l, that is 146% higher than the average (57 IU/l). All values were normally distributed except bilirubin, potassium, chloride, CPK, and gamma GT. There were no significant differences between male and female serum chemistry values.

### 4.3 PREVALENCE OF ANTIBODIES

Prevalence of antibodies against selected organisms were determined in serum from 47 adult kit foxes (26 female, 21 male) and 8 juveniles ( $\leq 9$  months old; 4 female, 4 male). Samples were collected between April 1989 and December 1990. Some serum samples were not large enough for all antibody tests to be performed. Antibodies were detected against five of the eight organisms tested. Complete immunology data for each fox are included in Appendix C.

Thirty-one of 43 (72.1%) samples from adult kit foxes tested positive for presence of antibodies against canine parvovirus (eight of which were recent exposures); 7 of 40 (17.5%) tested positive for toxoplasmosis antibodies; 8 of 41 (19.5%) tested positive for antibodies

against canine distemper virus; 8 of 38 (21.1%) tested positive for antibodies against one or more of the five *Leptospira* serotypes studied; and 10 of 37 (27.0%) tested positive for antibodies against canine hepatitis virus. Antibodies were detected in only one of eight samples from juvenile kit foxes; female fox #4182 had a titer of 1:100 for antibodies against two of the *Leptospira* serotypes (canicola and grippityphosa). Antibodies against the organisms that cause brucellosis, coccidioidomycosis, and sylvatic plague were not detected in any samples (number of samples tested = 46, 43, and 49, respectively).

## 5. DISCUSSION

The average hematological values for adults were all within normal ranges reported for adult San Joaquin kit foxes in Kern County (McCue and O'Farrell 1987). Only one fox (female #4129) had values outside of the normal ranges calculated for the Camp Roberts kit fox population that suggested an abnormal condition. This fox had low values in three hematological parameters: HGB (12.1 g/dl), RBC count ( $6.43 \times 10^6/\text{ul}$ ), and PCV (36.6%). These values indicate that the fox had a normocytic, normochromic anemia (i.e. the total number of RBC's and amount of hemoglobin were low, but the RBC's present were of normal size and hemoglobin concentration). Potential causes are chronic disease, infection, or tumors.

Male kit foxes inhabiting Camp Roberts had significantly higher WBC and neutrophil counts, and a lower percent of lymphocytes than female kit foxes. No differences in these parameters were reported for kit foxes in Kern County (McCue and O'Farrell 1987). Smith and Rongstad (1980) reported that male coyotes had significantly higher WBC counts than females. The difference was attributed to the more aggressive nature of males, resulting in higher stress and increased muscle activity (Smith and Rongstad 1980). The higher WBC count probably resulted from neutrophilia caused by splenic contractions during capture (Smith and Rongstad 1980). Smith and Rongstad (1980) also found that male coyotes had higher neutrophil counts and lower lymphocyte percentage than females (although the differences were not significant).

The average values for all serum chemistry parameters fell within normal values exhibited by San Joaquin kit foxes in Kern County (McCue and O'Farrell 1992). Only one fox had a serum chemistry value excluded as an outlier. Female fox #4157 had an alkaline phosphatase concentration of 140 IU/l, whereas the mean concentration of all of the other samples was 57 IU/l. The fox also had an elevated ALT concentration (396 IU/l), that along with the high alkaline phosphatase concentration, suggests liver disease. However, the gamma GT concentration, an indicator of acute liver disease, was normal.

Three foxes had serum chemistry values outside of the normal ranges calculated for the Camp Roberts kit fox population that suggested an abnormal condition. Female fox #3213 had an elevated glucose concentration (335 g/dl) which suggests the possibility of stress response. Female fox #3390 had slightly elevated creatinine (with high normal BUN) that suggests mild renal disease (decreased kidney perfusion, kidney disease, or post-kidney urinary tract obstruction). Female fox #4153 had elevated concentrations of AST (836 IU/l), CPK (6,358 IU/l), and LDH (581 IU/l). These values suggest either muscle damage or liver disease. The more specific liver enzymes ALT and gamma GT are not elevated, making the probability of muscle damage more likely.

The average concentration of ALT from Camp Roberts adult foxes (179 IU/l) is lower than those from Kern County (217 IU/l, SD = 112.9, n = 63) (McCue and O'Farrell 1992). ALT is an enzyme located within hepatocyte cells in the liver that leak after damage occurs in the cell membrane. The increased ALT suggests that the foxes in Kern County may have an increased incidence of hepatic damage. The average glucose concentration in Camp Roberts samples (185 mg/dl) is higher than reported in Kern County (129 mg/dl, SD = 28.8, n = 61).

This may be related to diet, stress, or simply treatment of samples after collection.

The occurrence of antibodies against the pathogens tested is similar to those reported for San Joaquin kit foxes in Kern County (McCue and O'Farrell 1988) with two exceptions. Over 17% of the samples collected during this study tested positive for antibodies against one or more of the *Leptospira* serotypes, whereas detectable levels were not found in any of 23 kit fox blood samples collected in Kern County (McCue and O'Farrell 1988). Exposure to *Leptospira* organisms is common in wild carnivores (Cirone et al. 1978). Antibodies against *Toxoplasma gondii* were detected in 17.5% of the samples tested from Camp Roberts, whereas McCue and O'Farrell (1988) did not detect any in 25 samples collected in Kern County. McCue and O'Farrell (1988) did detect antibodies against *T. gondii* in 2 of 10 samples collected from kit foxes on the Elkhorn Plain in San Luis Obispo County, however.

Among the five foxes which had hematology or serum chemistry values indicating an abnormal condition, all had titers against canine parvovirus, three of which (female foxes #4129, #3390, and #4153) were recent exposures. Female fox #3390 also had antibodies against distemper and the *hardjo* *leptospira* serotype.

While the prevalence of antibodies to the rabies virus was not tested, the virus was known to be present in the Camp Roberts kit fox population. In early 1990, two kit foxes were found dead due to rabies (Standley et al. 1992). The only other known occurrence of rabies in kit foxes was in 1989 when a rabid desert kit fox was found in Death Valley National Monument (Nancy Hagerman, National Park Service, pers. comm.). Raccoons and striped skunks were regularly captured while trapping for kit foxes at Camp Roberts (EG&G/EM 1991), and both are known vectors of rabies. San Luis Obispo County had the highest incidence of wildlife rabies cases of all counties in California during this study (Barrett 1990, Schultz and Barrett 1991, Reilly and Mangiamele 1992).

## **6. CONCLUSIONS**

The hematological and serum chemistry values found in San Joaquin kit fox at Camp Roberts are similar to values reported for kit foxes in other locations. There were indications that individual foxes had ongoing infections, liver and kidney disease, and muscle damage. Serological tests indicated that pathogens of at least five infectious diseases are present on Camp Roberts: leptospirosis, canine parvovirus, toxoplasmosis, infectious canine hepatitis, and canine distemper. There was no indication of the presence in the kit fox population of the pathogens that cause brucellosis, coccidioidomycosis, or sylvatic plague. It should be noted that while a positive titer against a pathogen suggests prior exposure, it does not necessarily indicate the presence of the disease. While rabies was known to cause the death of two radiocollared kit foxes between November 1988 and September 1991 (Standley et al. 1992), other infectious or noninfectious disease processes may have been the ultimate cause of deaths attributed to predation or unknown causes.

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Table 1. Hematologic values of adult San Joaquin kit fox at Camp Roberts, 1989. Separate information is shown for those values that differed significantly between sexes.

PARAMETER	SAMPLE SIZE (N)	MEAN (X)	STANDARD ERROR (SE)	NORMAL RANGE (X " 2 SD)
RBC (# x 10 <sup>6</sup> /mm <sup>3</sup> )	18	9.03	0.211	7.24 - 10.82
HGB (g/dl)	18	15.9	0.38	12.7 - 19.2
PCV (%)	18	51.1	1.41	39.2 - 63.1
MCV (fl)	18	61	0.63	50.8 - 61.6
MCH (pg)	18	17.7	0.18	16.2 - 19.2
MCHC (%)	18	31.4	0.38	28.2 - 34.6
Total WBC (# x 10 <sup>3</sup> /mm <sup>3</sup> )				
Male	8	9.3	0.83	4.6 - 14.0
Female	10	6.2	0.81	1.1 - 11.3
Neutrophils (#/mm <sup>3</sup> )				
Male	8	8037	752.5	3780.3 - 12293.9
Female	10	5012	640.8	959.9 - 9064.9
(%)	18	84	1.3	72.4 - 95.2
Lymphocytes (#/mm <sup>3</sup> )	18	751	89.2	0 - 1508.1
(%)				
Male	8	8	1.5	0 - 16.0
Female	10	13	1.7	2.4 - 24.0
Monocytes (#/mm <sup>3</sup> )	18	332	53.6	0 - 786.1
(%)	18	4	0.4	0.8 - 7.2
Eosinophils (#/mm <sup>3</sup> )	18	65	13.9	0 - 183.3
(%)	18	1	0.2	0 - 2.9
Basophils (#/mm <sup>3</sup> )	17	0		
(%)	17	0		

Table 2. Serum chemistry values of adult San Joaquin kit fox at Camp Roberts, 1989.

PARAMETER	SAMPLE SIZE (N)	MEAN (X)	STANDARD ERROR (SE)	NORMAL RANGE (X " 2 SD)
AST (IU/l)	17	266	47.1	0 - 654.9
ALT (IU/l)	17	179	21.6	0.9 - 357.4
Bilirubin (mg/dl)	17	0.2	0.02	0.04 - 0.29
BUN (mg/dl)	17	37	2.9	12.9 - 60.6
Creatinine (mg/dl)	17	1.2	0.09	0.47 - 1.95
BUN:Creatinine Ratio	17	31.7	2.80	8.65 - 54.83
Cholesterol (mg/dl)	17	157	4.3	121.5 - 193.1
Alkaline Phosphatase (IU/l)	16	57	3.5	28.7 - 86.0
Glucose (mg/dl)	17	175	15.7	45.2 - 304.6
Calcium (mg/dl)	17	8.8	0.07	8.23 - 9.32
Phosphorous (mg/dl)	17	5.4	0.20	3.75 - 7.08
Calcium:Phosphorous Ratio	17	1.7	0.07	1.09 - 2.26
Total Protein (g/dl)	17	5.3	0.10	4.52 - 6.09
Albumin (g/dl)	17	3.0	0.06	2.50 - 3.51
Globulin (g/dl)	17	2.3	0.08	1.65 - 2.95
Albumin:Globulin Ratio	17	1.3	0.05	0.90 - 1.75
Sodium (mEq/l)	17	151	1.2	141.8 - 160.9
Potassium (mEq/l)	17	4.5	0.09	3.80 - 5.20
Sodium:Potassium Ratio	17	33.8	0.65	28.49 - 39.15
Chloride (mEq/l)	17	120	0.9	112.4 - 127.8
Carbon Dioxide (mEq/l)	17	25	0.6	20.8 - 30.1
Creatine Phosphokinase (IU/l)	17	1796	461.4	0 - 5600.5
Gamma GT (IU/l)	16	5	1.8	0 - 19.7
Uric Acid (mg/dl)	17	0.4	0.07	0 - 0.99
Lactic Dehydrogenase (IU/l)	17	282	33.7	4.8 - 559.8