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HEMATOLOGY AND SERUM BIOCHEMISTRY VALUES OF DUSKY-FOOTED WOOD RAT (*NEOTOMA FUSCIPES*)

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ABSTRACT: Serum chemistry values and complete blood counts were determined for 36 wild dusky-footed wood rats (*Neotoma fuscipes*) from Sonoma and western Yolo County, California (USA) in summer 1999 and spring 2001. All wood rats had adequate body condition and were hydrated. Many hematologic and biochemical values were comparable to those for house rat (*Rattus rattus*). There were differences between wood rats tested immediately after capture (those from Yolo County) and after a week of habituation in the laboratory (Sonoma County). Significant differences were noted in red blood cell counts, hemoglobin, hematocrit, neutrophil:lymphocyte ratio, glucose, alanine transaminase, aspartate aminotransferase, and alkaline phosphatase values. The neutrophil:lymphocyte ratio may have been iatrogenically modified in the wood rats tested immediately after capture by stress-induced neutrophilia and lymphopenia. Eosinophilia may have been associated with parasites such as botflies in four individuals, and hyperglycemia in three individuals could have been associated with stress. The cause of elevated enzymes in the animals tested after laboratory habituation is unclear. The hematologic and biochemical values of these apparently healthy wood rats provide valuable baseline information for use in further medical studies performed with this species.

Key words: Clinical biochemistry, dusky-footed wood rat, hematology, *Neotoma fuscipes,* reference intervals.

INTRODUCTION

The dusky-footed wood rat (*Neotoma fuscipes*) is a large rodent which is an important natural host for several vector-borne zoonotic infections including *Ehrlichia phagocytophila* sensu lato (Nicholson et al., 1999; Foley et al., 2002) and *Borrelia burgdorferi* sensu lato (Brown and Lane, 1996). This species is common in mixed evergreen forest with dense understory and chaparral, sclerophyll woodland, and riparian woodland from Oregon through California (USA) to northern Baja California (Mexico). Laboratory studies to evaluate infected individual woodrats are more informative when results are analyzed in the context of normal values. However, published normal values for this species are not available. This paper presents the hematologic and serum biochemistry values for 36 apparently healthy field-collected adult wood rats from northern California.

MATERIALS AND METHODS

Twenty-one dusky-footed wood rats were trapped in spring 2001 in chaparral with oak in western Yolo County, California (38°53′18″N, 122°14′11″W). Large aluminum folded traps (Sherman, Tallahassee, Florida, USA) were baited with grain and peanut butter and padded with clean cotton and placed on the ground in the evening in an area inhabited by woodrats. At sunrise, animals were removed from the traps for anesthesia.

Fifteen dusky-footed wood rats were trapped in summer 1999 in a wooded area approximately 8 km north of Sonoma, California (38°17′31″N, 122°27′25″W). The soil at this site formed on volcanic bedrock with perennial water and the plant community consisted of mixed evergreen forest, with *Quercus agrifolia, Arbutus menziesii,* and *Umbellularia californica,* and an understory of *Rhus toxicodendron,* ferns, and annual grasses. Animals were trapped as described above; animals in occupied traps were transported by automobile 1.5 hr to individual cages at the University of California (Davis, California). Minimum noise, human exposure, and handling were employed to minimize capture stress and excitement. The animals were allowed to habituate to the labo-

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ratory environment in wire mesh cages (22×22×8 cm) with visual and olfactory contact with another wood rat for 1 wk before blood was sampled. Animals were fed commercial rodent feed. Ambient room temperature was 26.6 C with a 12 hr light/dark cycle supplied by artificial light.

To obtain blood samples, all wood rats were prompted to enter a glass mason jar containing a methoxyflurane (Pittman Moore, Inc., Washington Crossing, New Jersey, USA)-soaked wick and held in the jar until unconscious, as determined by failure to respond to gentle tactile stimuli. Prior to sampling, the clinical status of all animals was subjectively assessed by physical examination. Body condition was determined by palpation for subcutaneous fat and hydration was classed as normal, 3–5% dehydrated, or >8% dehydrated based on skin turgor, gingival mucosal moisture, capillary refill time, and orbital depression. Once anesthetized, approximately 1 ml of whole blood was obtained via retro-orbital placement of an ethylenediaminetetetraacetic acid (EDTA)-coated glass hematocrit tube. Half of the sample was collected into an EDTA tube for complete blood count and the remaining 0.5 ml was collected into a sterile tube with no anticoagulant for separation of serum for biochemistry. Blood samples were analyzed at the University of California Davis, Veterinary Medical Teaching Hospital. Automated cell counting was performed using a Serono Baker 9000 instrument (Biochemical Immunosystems, Allentown, Pennsylvania, USA). Red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), and mean cell volume (MCV) were directly measured, whereas hematocrit (Hct), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated by the instrument. Differential cell counts were performed manually from thin Wright-stained blood smears. Serum was separated from clotted cells by centrifugation at 2,000×G for 10 min. Biochemical analyses were performed on serum using a Hitachi 717 automated analyzer (Boeringer-Mannheim Corp., Indianapolis, Indiana, USA).

Data were maintained in a spreadsheet (Excel, Microsoft, Redmon, Washington, USA) and data analysis was performed using statistical software ("R", The R-Development Core Team, http://www.r-project.org/), with cutoff of $P \leq 0.05$ determining statistical significance. The mean, median, and standard deviation were calculated for each parameter and any outliers (values that differed from the mean by greater than three standard deviations) were excluded from data summaries (Lumsden and Mullen, 1978; Lumsden et al., 1979). For each parameter, a Shapiro-Wilk test was performed after exclusion of outliers to determine whether the results were normally distributed (Royston, 1982). A subset of normally distributed hematologic parameters was constructed and analyzed by multiple analysis of variance (MANOVA) to test the null hypothesis that there were no significant differences in these values based on gender or treatment (laboratory vs. immediate testing). The MANOVA was performed to control for problems of correlations among parameters. However, in order to evaluate each parameter, a two-way replicated ANOVA was performed for each normally distributed hematologic parameter and Wilcoxon rank sum test performed for non-normally distributed parameters, to evaluate whether that particular parameter differed by gender, treatment, or interaction. Likewise, ANOVA or Wilcoxon rank sum test was performed to evaluate biochemical parameters separately.

RESULTS

Physical examination of 36 field-caught mature woodrats indicated adequate body condition and no evidence of dehydration. Hematologic profiles were performed for 34 animals and summarized in Table 1. Normally distributed parameters included RBC count, Hg, Hct, MCV, MCH, MCHC, neutrophil numbers, and platelet numbers. Platelet numbers could not be evaluated in animals tested immediately after capture due to inadequate sample size. In animals habituated to the laboratory, platelets were adequate and not clumped in any samples. Values ranged from a low of 449,000 to a high of 933,000/μl, compared to established values for house rats (*Rattus rattus*) ranging from 500,000–1,300,000/μl (Harkness and Wagner, 1989; Hillyer and Quessenberry, 1997). The RBC counts, Hg, and Hct tended to be slightly lower in immediately tested individuals compared with those held in the laboratory. These parameters tended to be lower than the minimum normal values for rats of 700,000/μl cells, 11 g/dl, and 36%, respectively.

One individual had 31,500 WBCs/μl, although this value did not classify as a true outlier. This individual did not have bands or other immature leukocytes, but did

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TABLE 1. Summary of hematologic parameters for wild-caught *Neotoma fuscipes*. The first row for each parameter contains values from animals from Sonoma, California held in captivity for 1 wk prior to testing, second row is animals from western Yolo County, California, tested immediately after capture, and the third row is all animals combined.

Hematologic parameter	N	Mean	Median	SD	Range
Erythrocytes ($t10^6/\mu l$)	14	8.78	9	1.15	6.92–11.21
	20	6.97	6.7	1.12	5.7–10.56
	34	7.72	7.4	1.45	5.7–11.21
Hemoglobin (g/dl)	14	12.24	12.3	1.22	10.0–14.0
	20	10.52	10.6	1.21	8.2–13.4
	34	11.23	11.2	4.47	8.2–14
Hematocrit (%)	14	39.94	40.7	4.85	30.9–48.9
	20	34.88	34.8	4.74	26.9–45.3
	34	36.96	36.6	5.35	36.9–48.9
MCV (fl)	14	45.57	45.1	2.15	41.7–48.9
	19	50.51	50.4	4.48	42.9–60.8
	33	48.41	47.4	4.39	41.7–60.8
MCH (pgm)	14	14.01	14.4	0.85	12.3–14.9
	20	15.20	15.4	1.16	12.7–17.4
	34	14.71	14.7	1.19	12.3–17.4
MCHC (g/dl)	14	30.75	31.0	1.30	28.2–32.4
	19	30.15	30.1	1.40	26.8–32.1
	33	30.40	30.5	1.37	26.8–32.4
Leukocytes (/μl)	14	11,421.0	9,500	7,358.6	4,400–31,500
	20	10,465.8	10,600	3,491.5	4,200–18,800
	34	10,858.0	10,250	5,345.0	4,200–31,500
Neutrophils (/μl)	14	3,999.0	3,474	3,395.0	1,152–14,175
	20	7,831.6	8,007	3,160.3	2,394–15,980
	34	6,253.4	5,894	3,736.0	1,152–15,980
Lymphocytes (/μl)	14	5,885.0	4,465	3,627.0	1,794–13,860
	20	1,967.4	1,816	1,006.8	715–5,040
	34	3,580.3	2,268	3,097.6	715–13,860
Monocytes (/μl)	14	706.09	541	484.6	188–1,575
	20	429.5	315	312.4	101–1,341
	34	543.3	429	410.0	101–1,575
Eosinophils (/μl)	14	516.9	489	496.9	47–1,890
	20	142.2	105	159.7	0–576
	34	296.5	120	383.4	0–1,890
Basophils (/μl)	14	288.3	288	265.3	0–852
	20	158.0	53	112.7	0–404
	34	170.5	108	212.5	0–852
Platelets (/μl)	14	651,786	653,000	144,295	449,000–933,000
	0	—	—	—	—
	14	651,786	653,000	144,295	449,000–933,000

have neutrophilia of 14,175 neutrophils/μl, lymphocytes within normal range, monocytosis of 1,575 monocytes/μl, and eosinophilia of 1,890 eosinophils/μl. Leukocyte numbers in the lower range were lower than the rat lower range of 6,000/μl. In wood rats tested immediately after capture, neutrophils exceeded lymphocytes in all individuals, with an overall ratio of 4.0:1 neutrophils:lymphocytes. However, in animals held in the laboratory, neutrophils exceeded lymphocytes in three individuals but the reverse was true in the remaining 11. The overall ratio in this group was 0.7:1 neutrophils:lymphocytes, more closely resembling the ratio in rats. No toxicity was detected in neutrophils but there were 1% bands in two individuals and 3% bands

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TABLE 2. Summary of serum biochemical parameters for wild-caught *Neotoma fuscipes*. The first row for each parameter contains values from animals from Sonoma, California held in captivity for 1 wk prior to testing, second row is animals from western Yolo County, California, tested immediately after capture, and the third row is all animals combined.

Serum chemistry parameter	N	Mean	Median	SD	Range
Sodium (mmol/l)					
	10	149.9	149	3.34	145–155
	0	—	—	—	—
	10	149.9	149	3.34	145–155
Potassium (mmol/l)	10	4.02	3.9	0.52	3.5–4.8
	0	—	—	—	—
	10	4.02	3.9	0.52	3.5–4.8
Chloride (mmol/l)	10	106.2	107	3.55	100–112
	0	—	—	—	—
	10	106.2	107	3.55	100–112
Calcium (mg/dl)	13	10.6	10.8	1.06	7.7–12
	21	9.0	9.3	1.1	5.6–10.2
	34	9.6	9.7	1.34	5.6–12
Phosphorus (mg/dl)	13	7.2	6.9	2.43	4–10.2
	21	7	6.4	2.70	3.2–13.6
	34	7.1	6.5	2.57	3.2–13.6
Creatinine (mg/dl)	13	0.3	0.3	0.12	0–0.4
	1	0.2	0.2	—	0.2
	14	0.27	0.3	0.11	0–0.4
Blood urea nitrogen (mg/dl)	13	20.6	21	6.21	10–32
	21	22.7	24	6.49	12–36
	34	21.9	24	6.37	10–36
Glucose (mg/dl)	13	135.0	133	27.00	94–196
	21	103.9	110	34.55	60–216
	34	116.0	116	34.9	60–216
Total protein (g/dl)	13	6.8	6.8	0.61	5.7–8.0
	20	6.8	6.9	0.90	4.8–8.0
	33	6.8	6.8	0.77	4.8–8.0
Albumin (g/dl)	13	3.4	3.4	0.80	1.2–4.2
	21	2.9	2.8	0.45	2.2–3.8
	34	3.1	3.1	0.60	1.2–4.2
Globulin (g/dl)	13	3.4	3.2	0.76	2.7–5.6
	21	3.8	3.9	0.99	1.8–5.6
	34	3.7	3.6	0.93	1.8–5.6
Alanine transaminase (IU/l)	13	11.2	11	5.07	1–22
	21	21.0	18	11.60	3–48
	34	17.2	15	10.65	1–48
Aspartate aminotransferase (IU/l)	13	134.0	112	72.00	55–329
	21	311.0	281	153.31	24–582
	34	245.0	198	154.05	24–582
Alkaline Phosphatase (IU/l)	13	103.0	92	55.20	46–245
	21	128.9	114	51.67	57–236
	34	119.0	104	53.80	46–245
γ Glutamyl transferase (IU/l)	13	0.46	0	0.52	0–1
	21	0.29	0	0.72	0–2
	34	0.35	0	0.65	0–2
Bilirubin total (mg/dl)	13	0.12	0.1	0.07	0–0.4
	21	0.24	0.2	0.13	0–0.4
	34	0.19	0.2	0.13	0–0.4
Cholesterol (mg/dl)	13	136.0	117	90.00	90–227
	21	121.4	118	36.49	70–218
	34	127.0	118	39.90	70–227

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in two others. Only one of the individuals with bands had neutrophilia. High eosinophil counts (735–1,890/μl) were detected in four woodrats but none of these individuals had absolute increases in leukocytes. There were no noted abnormalities of morphology, color, or size. Results of MANOVA indicated significant overall differences in normally distributed hematologic parameters based on treatment ($P=0.0001$) and the interaction of treatment and gender ($P=0.007$) but not gender alone ($P=0.38$). Significant differences between treatment groups were detected by ANOVA or Wilcoxon rank sum test for RBC counts ($P=0.9\times10^{-4}$), Hg ($P=4.1\times10^{-4}$), Hct ($P=0.005$), MCV ($P=9.2\times10^{-4}$), MCH ($P=0.003$), neutrophils ($P=3.9\times10^{-4}$), and lymphocytes ($P=9.5\times10^{-5}$), but not MCHC or WBC, monocyte, eosinophil, or basophil counts.

Thirty-four woodrats were sampled for serum biochemistry although electrolytes were evaluated only in animals held in captivity for 1 wk before sampling (Table 2). Normally distributed parameters included sodium, potassium, chloride, calcium, phosphate, blood urea nitrogen (BUN), glucose, total protein, albumin, and globulin. Sodium and chloride values were similar to those for rats with no outliers; however all potassium values were lower than the low end normal value for rats of 5.2 mmol/l. Most values for calcium and phosphorus were unremarkable and values were comparable between laboratory-held and immediately-tested animals.

The parameters associated with kidney function, BUN and creatinine, were within rat normal values. No individual had markedly elevated renal parameters. While the BUN tended to be higher for wood rats tested immediately after capture than for animals held in captivity for a week; the reverse was true for creatinine. For serum glucose, mean and median values were comparable to rats, although it was common for wood rats to have somewhat higher levels, approaching 200 g/dl. Mean and median glucose were higher in wood rats tested immediately, although the upper range was comparable between the groups. Albumin levels were similar between groups of wood rats as were globulin, although all wood rats tended to have somewhat high globulin compared with rats. The enzyme activities of alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were much higher in animals tested after being held in captivity than those tested immediately, although this pattern was not observed in γ glutamyl transferase (GGT) or cholesterol. Values for AST in particular, and to a lesser degree cholesterol, from the wood rats were much higher than for rats.

Significant differences between treatment groups were detected by ANOVA or Wilcoxon rank sum test for calcium ($P=1.3\times10^{-4}$), glucose ($P=0.01$), bilirubin ($P=0.004$), AST ($P=3.5\times10^{-4}$), ALT ($P=0.002$), glucose ($P=0.003$), and albumin ($P=0.02$), but not BUN, phosphate, creatinine, globulin, cholesterol, alkaline phosphatase, GGT, or total protein.

DISCUSSION

Hematologic and biochemical values of 36 apparently healthy wild-caught adult dusky-footed wood rats are presented as baseline information for use in further medical studies performed with this species. Collection and testing techniques were straightforward and sufficient volume of blood for complete profiles was readily available from most individuals. While the data from additional individuals would help provide confidence in appropriate normal limits for these parameters, the present data document the similarity between wood rats and rats in most parameters, with several important distinctions. It is important to use healthy animals in the establishment of "normal values"; however, it may be difficult to determine the health of individuals when studying field-caught wild species. However, all of the animals tested in this study appeared

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healthy, were normally hydrated, and were in good body condition.

Unfortunately, there were at least three treatment differences between the Sonoma and Yolo wood rats, so any detected differences could not be ascribed as to cause. Treatment differences included the time at which animals were tested (immediately after capture or after a week of habituation in the laboratory), the geographic source of origin, and the subspecies, with animals from Sonoma in the subspecies *N. fuscipes monochroura* and animals from Yolo in the subspecies *N. fuscipes fuscipes.* In order to evaluate the source of differences between the groups, more testing would obviously need to be performed; however the objective of the present paper was simply to present data from field-caught *N. fuscipes.*

Only one individual had significantly high WBC counts; perceived differences in neutrophil counts between the treatment groups may actually be insignificant given the generally wide reference range of neutrophil numbers in many mammalian species. There was moderate eosinophilia in three individuals, which is often reported in free-ranging wildlife due to parasitic infestation (Feldman et al., 2000). Full parasitologic evaluations were not performed on animals in the laboratory, although two of the Sonoma wood rats had botfly larvae embedded within their skin. Once the larvae emerged, the lesions healed well.

Neutrophils were consistently favored in all of the immediately-tested individuals, while lymphocytes outnumbered neutrophils in most but not all of the animals tested after a week in the laboratory. Typically in rodents, including gerbils, hamsters, mice, and rats (Hillyer and Quessenberry, 1997), lymphocytes outnumber neutrophils, suggesting that the wood rats tested after a week in the laboratory may have had a more typical leukogram for this species. In the immediately tested wood rats, neutrophil numbers were higher and lymphocyte numbers lower than in most of the laboratory-tested wood rats. Possible causes of relative neutrophilia include inflammation, stress with steroid response, and exercise with epinephrine release, of which the latter two are almost certainly relevant. The relative absence of inflammation is supported by the lack of left shift and low magnitude of neutrophilia, while endogenous steroid response is consistent with the relative lymphopenia. These findings need further evaluation to determine whether other differences between the groups such as the anesthetic agent or subspecies are relevant, but may suggest that a more accurate assessment of the leukogram would likely be acquired if the animal is given time to recover from a potentially traumatic trapping experience.

In the serum biochemical analysis, the most notable results were slightly low potassium values, higher BUN:creatinine ratios in immediately-tested wood rats than laboratory tested wood rats, slightly elevated glucose, slightly elevated globulin, and distinctly elevated ALT, AST, and ALP in laboratory-tested wood rats. The reason for the slightly low potassium is unknown. The slightly elevated BUN in immediately-tested wood rats was not statistically significant but, if it were real, could be due to extra-renal or renal causes. It is most plausible that the mild azotemia would be extrarenal given the lack of concomitant elevated creatinine. The most likely explanation is relative hemoconcentration in this group due to mild dehydration and stress, although this could not be determined without further testing such as urinalysis. There were three wood rats with a glucose level of approximately 200 mg/dl (two tested in the field and one in the laboratory), likely due to stress and steroid production. However, these three animals did not have particularly marked neutrophilia. The fact that none had hypoglycemia indicates that feeding in the laboratory was adequate and that the lack of feed during the capture period was not sufficient to alter the parameter. The elevated globulin level probably reflects antigenic

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stimulation due to the many pathogens encountered by free-living wildlife. There were significant elevations in three of the liver enzyme activities of laboratory-tested wood rats, and the possible role of the anesthesia is important to consider.

In summary, this study presents baseline hematologic and biochemical information for apparently healthy adult wild dusky footed wood rats. While comparisons between the groups are preliminary, the results suggest a testable hypothesis that capture stress can induce changes in the leukogram and that such changes may resolve after carefully managed laboratory habituation. These data will allow for future comparison with wood rats and wild-caught rodents of other species, and for better assessment of the impact of laboratory manipulations, including experimentally induced infectious diseases, on individual dusky-footed wood rats.

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