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Hematology and Plasma Chemistry Reference Intervals for Mature Laboratory Pine Voles (*Microtus pinetorum*) as Determined by Using the Nonparametric Rank Percentile Method

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Plasma biochemical and hematologic values are important parameters for assessing animal health and experimental results. Although normal reference values for many rodent species have been published, there is a dearth of similar information for the genus *Microtus*. In addition, most studies use a mean and standard deviation to establish reference intervals, but doing so is not the recommendation of the Clinical and Laboratory Standards Institute (formerly the National Committee on Clinical Laboratory Standards) or the International Federation of Clinical Chemistry and Laboratory Medicine. The purpose of this study was to establish normal reference parameters for plasma biochemistry and hematology in mature pine voles (*Microtus pinetorum*) by using the nonparametric rank percentile method as recommended by the 2 laboratory medicine organizations mentioned. Samples of cardiac blood from a closed colony of pine voles were collected at euthanasia and evaluated under rodent settings on 2 automated hematology analyzers from 2 different manufacturers and on the same type of automated biochemistry analyzer. There were no sex-associated clinically significant differences between the sexes; younger animals had a lower hematocrit, higher mean corpuscular volume, and lower mean corpuscular hemoglobin concentration than did older animals. Only platelet counts differed when comparing hematologic values from different analyzers. Relative to rats and mice, pine voles have a lower mean corpuscular volume and higher red blood cell count, higher blood urea nitrogen, much higher alanine aminotransferase, and lower glucose and phosphorous concentrations. Hematology and plasma biochemical results obtained in this study are considered representative for healthy adult laboratory pine voles under similar environmental conditions.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; WBC, white blood cell

Rodents belonging to the family Microtidae are often animal models used in behavioral, physiologic, and ecologic studies. Pine voles (*Microtus pinetorum*) are monogamous and possess several associated behavioral traits, such as a cooperative breeding system, the formation of pair bonds, and biparental care. These features contrast sharply with meadow voles (*Microtus pennsylvanicus*) and motane voles (*Microtus montanus*), which are polygamous and solitary. Microtine rodents, particularly *Microtus pinetorum*, historically have served as a model for studies in behavioral neuroscience and endocrinology.[3,14,19,21,32,37,38] More recently, pine voles have been evaluated as a model for infection with *Rickettsia rickettsii*, the causative agent of Rocky Mountain Spotted Fever.[15]

Laboratory pine voles have a lifespan in the laboratory that typically exceeds 18 mo and may have a total lifespan exceeding 3 y. Pine voles are relatively easy to maintain in a laboratory setting and can be housed in translucent or transparent mouse caging because their physical size (20 to 28 g) is roughly comparable to that of a mouse.[36] This species of vole reaches sexual maturity by 4 mo of age, forms monogamous pair bonds, and is an induced ovulator. Pine voles have an approximately 28-d gestation period, produce 1 to 4 pups per litter, and demonstrate cooperative breeding and care of young.

Although considerable scientific literature addresses the behavioral and reproductive features of pine voles, information on the normal hematologic and plasma biochemical features of this species is sparse. Some limited hematologic information is available for meadow voles[16,18] and prairie voles (*Microtus ochrogaster*)[20] but not for pine voles. In addition, most reference intervals for laboratory species have been determined by using the mean and standard deviation of a reference group. However, this method is not recommended by the Clinical and Laboratory Standards Institute (formerly the National Committee on Clinical Laboratory Standards) or the International Federation of Clinical Chemistry and Laboratory Medicine. The rationale underlying this stance is that many biological analytes, particularly biochemical values such as creatinine and bilirubin, are not normally distributed around a central mean. These data are

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often log-normal and left-skewed: values are clustered closer to 0 rather than being distributed around a mean greater than 0. Therefore, reference intervals calculated by using the mean plus and minus 2 standard deviations do not adequately represent the central 95% of the reference population and, in some cases, include values less than 0 or nonphysiologic values greater or less than those actually measured. The current preferred method is the rank percentile method, which does not rely on normality of data to establish a reference interval.[13,33,34,35] The purpose of this study was to establish clinically relevant normal hematological and plasma biochemical values in the adult laboratory pine vole by using methods recommended by the by the Clinical and Laboratory Standards Institute and International Federation of Clinical Chemistry and Laboratory Medicine, with the intent that this information would be useful as a reference for clinicians, laboratory animal veterinarians, and biomedical research scientists.

Materials and Methods

Animals. A total of 112 pine voles were used in this study and were descendants of voles originally trapped in Henderson County, NC. No new animals had been introduced into the colony for more than 6 y prior to this study. Animals were housed according to standard operating procedures established at the Biological Resources Facility (College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC) in accordance with regulatory standards promulgated by the Animal Welfare Act[1] and the *Guide for the Care and Use of Laboratory Animals*.[29] All animal use activity was approved by the Institutional Animal Care and Use Committee of North Carolina State University.

Feed (diet 5015, Purina Mills International, St Louis, MO) and water were provided ad libitum. Animals were maintained on a 14:10-h light:dark cycle in a room set at 21 °C. Animals used in the study were adults, not active breeders, and housed in 18 × 29 × 12.5 cm polypropylene cages with siblings on corncob bedding and an autoclaved 1- to 3-cm diameter twig of hardwood (typically applewood) for enrichment.

Colony health was monitored quarterly by evaluation of serologic responses to a standard battery of mouse and rat pathogens via a dirty-bedding sentinel program, although these assays have not been validated specifically for microtine rodent samples. Serum samples from outbred female CD1 mice and outbred female Sprague–Dawley rats were evaluated quarterly by the Research Animal Diagnostic Laboratory (University of Missouri–Columbia, Columbia, MO) for serologic antibody responses to mouse hepatitis virus, mouse parvovirus, mouse minute virus, Sendai virus, Theiler mouse encephalitis virus, murine rotavirus, *Mycoplasma pulmonis*, rat coronavirus, pneumonia virus of mice, and rat parvovirus. Sentinels also were evaluated quarterly for ectoparasites and endoparasites. A review of sentinel results for the last 5 y did not reveal seroconversion or positive results to any of the named pathogens. A single PCR evaluation of feces for *Helicobacter* species revealed that 2 of the 4 samples were positive for *Helicobacter rodentium*.

Procedure. Blood samples were obtained from apparently healthy, clinically normal adult voles by cardiocentesis immediately after euthanasia with carbon dioxide. All blood samples were collected with a 20-gauge needle on a 1-ml syringe between the hours of 1400 and 1700. Approximately 200 µl of blood was collected for hematology and 400 µl for clinical chemistry, for a total of 600 µl. Samples for hematology were placed immediately in 600-µl plastic tubes containing lithium heparin (Microtainer, Becton Dickinson, Franklin Lakes, NJ), whereas samples for clinical chemistry were placed immediately in 600 µl plastic plasma separator tubes with lithium heparin (Microtainer, Becton Dickinson) and promptly centrifuged. Heparinized whole blood and centrifuged plasma samples were stored at 4 to 6 °C and placed for transportation in a standard insulated box with a cold pack. All samples for hematologic and plasma chemical analyses were collected by overnight courier at 1800 h (that is, within 3 h of harvest from the animal), and delivered to an independent veterinary reference laboratory (AnTech Diagnostics Laboratories, Farmingdale, NY, and Smyrna, GA). Hematology results were determined by using the rodent settings on either automated hematology analyzer A (Advia 120, Bayer Diagnostics, Tarrytown, NY) or B (Cell-Dyne 3500, Abbott Laboratories, Abbott Park, IL). The choice of hematology analyzer was based on the scheduling parameters dictated by AnTech: samples collected most weekdays were sent to the laboratory in NY (which used the Advia 120), whereas those obtained on Fridays and weekends were sent to the laboratory in GA (which used the Cell-Dyne 3500). Plasma chemistry results were analyzed on the same type of automated biochemistry analyzer (Hitachi 747–200, Hoffman–LaRoche, Basel, Switzerland) regardless of location. Results were received via fax by 0730 the following morning, within 17.5 h of collection.

After blood withdrawal, 50 animals underwent detailed gross necropsies; 12 of these animals were evaluated histologically by a board-certified veterinary pathologist to identify any potential pathologic lesions.

Data analysis. All statistical analyses were performed by using SAS version 9.1 (SAS Institute, Cary, NC). All samples were included in the initial statistical analysis. Platelets values reported as 'too numerous to count' or '>1000' were included as 1000×10^9/L for the purpose of statistical analyses, as this value is the maximum detectable by hematology analyzers. Excluding very elevated platelet values would have resulted in misrepresentation of the platelet counts, because doing so would bias the results toward low values. In 1 report (generated on analyzer B), the platelet count was reported as 'adequate'; we substituted the average of all platelet values generated on that analyzer in statistical analyses. Histograms of each parameter were visually inspected for normality and categorized as Gaussian (normal), log-normal, or linear. Log-normal distributions were subcategorized based on skewing left or right. Student *t* tests (2-sided, $\alpha = 0.05$) were used to compare all biochemical parameters between the 2 biochemical analyzers at different locations, hematologic parameters between analyzers A and B, and sex (male or female). An F test was used to test for equality of variances for all prior comparisons. If the variances were unequal ($\alpha < 0.05$) then the Satterthwaite *t* test was performed; otherwise, variances were pooled. A simple linear correlation analysis, the CORR procedure, was performed to test for correlation between age (in days) and measured parameters. The CORR procedure is a statistical procedure for numeric random variables that computes Pearson correlation coefficients, 3 nonparametric measures of association, and the probabilities associated with these statistics.

The central 95% and 50% interfractile reference intervals were determined by using the nonparametric rank percentile method according to the guidelines of the Expert Panel on Theory of Reference Values of the Internal Federation of Clinical Chemistry.[33] The mean, median, and standard deviation were calculated with the AVERAGE, MEDIAN, and STDEV functions of Excel 2003 (Microsoft, Redmond, WA). The 0.025, 0.25, 0.75, and 0.975 percentile value for each parameter was generated with the PERCENTILE function of Excel 2003 (Microsoft). Briefly, all

values are ranked from smallest to largest, with consecutive rank numbers associated to equal values. The rank number of the desired percentile is calculated as $\% \times (n+1)$, where n is the total number of values. The reference limit is the value corresponding to the rank number if the rank is an integer. If the calculated rank is not an integer, the value is determined by interpolation between the 2 reference values above and below the calculated rank. This method is valid regardless of the data distribution pattern, although the results can be misleading if the number of subjects is less than 50.[33,35]

Results

A total of 112 animals, 65 female and 47 male, were used. Age and weight were normally distributed, with means of 353 d (SD, 160 d; range, 47 to 1254 d; median, 355 d) and 21.9 g (SD, 3.4 g; range, 16.0 to 33.4 g; median, 21.7 g). Neither sex and age or sex and weight were significantly associated. Blood for both biochemistry and hematology was collected from 78 voles. The remaining 34 animals yielded insufficient blood for both hematology and biochemistry; therefore 9 voles were evaluated only for biochemistry and 25 voles were only evaluated for hematology. Thus a total of 87 samples were collected for biochemical analysis and 103 for hematologic evaluation. Of the hematology samples, 24 were analyzed in GA (on analyzer B) and 79 in NY (on analyzer A). Of the 87 biochemistry samples, 67 were evaluated in NY, and the remaining 20 were evaluated in GA.

Results were unavailable for glucose and phosphorous for 1 male vole and for alanine aminotransferase (ALT), albumin, globulin, and total bilirubin in 1 female vole. One outlier was 3 times (300%) greater than the next lowest measurement and was removed from the reference intervals of total bilirubin after visual inspection of the histogram; all other biochemical and hematologic values were within 1 SD of the mean for this 13-mo-old female vole. All of the biochemistry results from a single 11-mo-old female vole were excluded from all further statistical evaluation and generation of reference intervals because its samples yielded values for 3 distinct biochemical analytes that were markedly elevated outliers (greater than 3 SD above the mean)—this animal therefore was presumed to be unhealthy.

Similarly, all the hematology results from one 14-mo-old male were excluded because the hematocrit was 1/3 less than the next lowest value, mean corpuscular volume (MCV) was 3 times greater than the next highest value, red blood cell (RBC) count was half the next closest value, and the animal was interpreted to have a marked regenerative anemia. However, its biochemical data all fell within less than 2 SD of the mean for the respective parameters and therefore were retained in the analyses. Of the remaining 102 hematology samples, 24 were performed on hematology analyzer B and the remaining 78 on hematology analyzer A. For 2 voles machine-generated hematology results were available, but a blood smear evaluation was not performed, and all leukocyte parameters were excluded. Further, 23 platelet values were accompanied by a comment regarding clumped platelets—4 (17% of all samples) of those samples were analyzed on hematology analyzer A and the remaining 19 (24% of all samples) on hematology analyzer B. Because the degree of clumping could not be evaluated and because the indicated platelet counts were scattered evenly throughout the data set (that is, did not skew toward low values), all were included in the analysis.

Visual examination of all the histograms did not reveal any noteworthy departures from normality, although several parameters showed nonsymmetrical distributions. After statistical analyses, no parameter was associated with results that differed significantly between sexes, and values from male and female voles were grouped for the generation of reference intervals. Biochemical analytes measured on the biochemistry analyzers in NY and GA did not differ statistically for any test; therefore biochemical values also were combined. However, platelet counts differed significantly ($P < 0.0001$, t and Wilcoxon tests) between samples assayed on the 2 hematology analyzers and therefore were evaluated separately. All other hematologic parameters were not significantly different between the 2 hematology analyzers and similarly were pooled. Descriptive and summary results of the hematology and biochemistry values, including 95% reference intervals, are presented in Tables 1 and 2, respectively.

Analytes that had a Gaussian distribution included RBC count, hemoglobin, mean corpuscular hemoglobin, glucose, blood urea nitrogen (BUN), total protein, albumin:globulin ratio, and phosphorous. Hematocrit, MCV, all leukocyte parameters, platelets measured as measured on hematology analyzer B, creatinine, globulin, total bilirubin, ALT, and alkaline phosphatase (ALP) had a left-skewed log-normal distribution, whereas mean corpuscular hemoglobin concentration (MCHC), albumin, and platelets as measured on hematology analyzer A had a right-skewed log-normal distribution. The BUN:creatinine ratio could not be classified as either Gaussian or log-normal, because it had a distinct bimodal distribution, possibly due to its nature as a fraction with 2 independent physiologic variables.

Linear regression revealed weak positive correlations between age and hemoglobin ($r = 0.22$, $P = 0.0276$), hematocrit ($r = 0.37$, $P = 0.0001$), and MCV ($r = 0.21$, $P = 0.0380$). There was a significant weak negative correlation between age and MCHC ($r = –0.29$, $P = 0.0037$).

None of the 50 animals that underwent detailed necropsies had any gross lesions. Histopathologic evaluation of 12 animals also did not yield evidence of abnormalities, and all of their data points fell within the established reference intervals. Unfortunately, the animals excluded from statistical analyses were not among those available for histopathologic evaluation. In particular, the 11-mo-old female with multiple biochemical abnormalities was not necropsied.

Discussion

Evaluation of health status requires comparison to known healthy individuals. However, hematologic and biochemical values for the pine vole, *Microtus pinetorum*, have not previously been published. The few published hematology and biochemistry results for other species of voles do not have 95% interfractile intervals, but rather state a mean and infrequently state a standard deviation. Currently, determination of interfractile intervals is the preferred method of reporting normal ranges, because it does not rely on a normal distribution of values for accuracy and cannot result in nonphysiologic values.[13,34,35] Blood collection techniques, instrumentation, and general methodology often are not declared or unavailable and vary between studies. Euthanasia procedure, site of collection, and the delay of as long as 17.5 h until analysis are particularly important and must be considered when using the results of the current study or comparing them with other studies.

A comparison of the reference interval calculated from our data by using the nonparametric rank-percentile method and that calculated by using the mean plus and minus 2 standard deviations as seen in the 2 tables demonstrates the strength of the former method to determine a representative central 95% for

Table 1. Combined nonparametric rank percentile reference intervals for pine vole (*Microtus pinetorum*) cardiac heparin-anticoagulated hematology results

	Unit	n	Distribution	Mean	Median	SD	2.5th–97.5th rank percentile	Mean – 2 SD to mean + 2 SD	25th–75th rank percentile
Red blood cells	$\times 10^{12}$/l	102	Gaussian	11.0	11.0	1.0	9.0–12.8	9.0–12.9	10.4–11.5
Hemoglobin	g/dl	102	Gaussian	15.0	15.0	1.1	12.8–17.0	12.8–17.1	14.2–15.9
Hematocrit	l/l	102	Log-normal (L)	40.8	40.2	4.3	34.8–49.1	32.3–49.4	37.3–43.3
MCV	fl	102	Log-normal (L)	37.5	36.0	5.0	32.5–48.0	27.5–47.4	34.3–39.0
MCH	pg	102	Gaussian	13.7	13.7	1.1	12.0–15.3	11.4–16.0	13.0–14.3
MCHC	g/dl	102	Log-normal (R)	36.9	37.6	2.6	31.3–40.3	31.6–42.1	35.8–38.4
White blood cells	$\times 10^{6}$/l	100	Log-normal (L)	4102	3700	1700	2048–8315	710–7494	3000–4900
PMN	$\times 10^{6}$/l	100	Log-normal (L)	1098	883	772	185–3284	(–445)–2642	536–1464
Bands	$\times 10^{6}$/l	100	Linear	0	0	0	0–0	0–0	0–0
Lymphocytes	$\times 10^{6}$/l	100	Log-normal (L)	2771	2662	1380	763–5720	10–5532	1850–3281
Monocytes	$\times 10^{6}$/l	100	Log-normal (L)	181	149	169	11–517	(–156)–519	80–224
Eosinophils	$\times 10^{6}$/l	100	Log-normal (L)	28	0	54	0–183	(–81)–136	0–36
Basophils	$\times 10^{6}$/l	100	Log-normal (L)	22	0	56	0–100	(–90)–133	0–26
Platelets—analyzer A	$\times 10^{9}$/l	78	Log-normal (R)	656	670	264	128–1000	127–1185	472–905
Platelets—analyzer B	$\times 10^{9}$/l	24	Log-normal (L)	370	334	167	116–695	36–705	275–480

(L), skewed left; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PMN, polymorphonuclear neutrophils; (R), skewed right

Table 2. Combined nonparametric rank percentile reference intervals for pine vole (*Microtus pinetorum*) cardiac heparinized plasma biochemistry results

	Unit	n	Distribution	Mean	Median	SD	2.5th–97.5th rank percentile	Mean – 2 SD to mean + 2 SD	25th–75th rank percentile
Glucose	mg/dl	85	Gaussian	100	105	32	28–158	36–164	82–120
Blood urea nitrogen	mg/dl	86	Gaussian	25	25	8	5–39	10–41	21–30
Creatinine	mg/dl	86	Log-normal (L)	0.2	0.2	0.1	0.1–0.6	(–0.1)–0.5	0.1–0.3
BUN:creatinine	ratio	86	Bimodal	166	135	101	18–369	(–36)–368	81–250
Total protein	g/dl	85	Gaussian	6.4	6.5	0.9	4.8–7.8	4.6–8.2	6.0–6.9
Albumin	g/dl	85	Log-normal (R)	4.0	4.1	0.8	1.3–5.0	2.5–5.5	3.9–4.5
Globulin	g/dl	85	Log-normal (L)	2.4	2.4	0.6	1.5–3.7	1.3–3.5	2.0–2.6
Albumin:globulin	ratio	85	Gaussian	1.8	1.8	0.5	0.7–2.7	0.8–2.8	1.5–2.1
Total bilirubin[a]	mg/dl	84	Log-normal (L)	0.2	0.1	0.1	0.1–0.3	0–0.3	0.1–0.2
ALP	U/l	86	Log-normal (L)	126	125	78	30–333	(–29)–281	78–150
ALT	U/l	85	Log-normal (L)	189	149	132	6–553	(–76)–453	111–237
Phosphorous	mg/dl	85	Gaussian	8.4	8.8	1.9	3.3–11.6	4.5–12.3	7.5–9.6

[a]1 outlier of 300% difference removed
ALP, alkaline phosphatase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; (L), skewed left; (R), skewed right

all values regardless of distribution. Leukocyte upper reference limits are similar, but the lower reference limit is much lower and is often a negative value when calculated using the mean minus 2 SD. The same is true for creatinine, the BUN:creatinine ratio, ALP, and ALT. The lowest measured total bilirubin was 0.1 mg/dl, yet the lower limit calculated using the mean minus 2 SD is 0, a nonphysiologic value. Similarly, the highest measured phosphorus concentration was 11.9 mg/dl, yet the upper reference limit calculated by using the mean plus 2 SD exceeded this threshold at 12.3 mg/dl, whereas the upper limit by using the rank percentile method is a more representative 11.6 mg/dl. Although the rank percentile method can be unreliable with fewer than 50 animals, it will provide a more representative and physiologically accurate interval of the central 95% of animals and cannot result in nonphysiologic values.[33,34]

The interactions of age, photoperiod, and androgen on hematocrit, RBC count and total protein have been documented in the common vole (*Microtus arvalis*)[10,11] as have seasonal changes in leukocyte count, hemoglobin and RBC count.[30,31] Other studies have shown that white blood cell (WBC) counts are dependent on the reproductive status and that nonreproductive males and females have similar WBC counts.[18] Several other studies have shown that small wild rodents, including voles, quickly develop hypoglycemia when fasted[27,28] and WBC counts can concurrently decrease.[27] Though age and sex differences were not detected in this study with the relatively small numbers of animals involved, there may be insufficient statistical power to detect subtle differences. Despite this, use of these reported intervals should be for fed animals kept under similar photoperiods and environmental conditions and when blood is collected in a similar manner.

Our plasma biochemistry results for BUN, ALP, bilirubin, ALT, total protein, and phosphorous overlap with those reported for prairie voles (*Microtus ochrogaster*; n = 52; isoflu-

rane anesthesia and lethal cardiocentesis) and meadow voles (*Microtus pennsylvanicus*; n = 11; isoflurane anesthesia and lethal cardiocentesis) with the exceptions of a higher upper limit of creatinine,[20] and generally higher total albumin measurements for prairie voles[20] and meadow voles (n = 29; ether anesthesia and lethal orbital sinus venipuncture).[1,2,20] One report of effects of alkaloid pesticide exposure in the meadow vole (*Microtus pennsylvanicus*; n = 15; euthanasia by cervical dislocation and immediate cardiocentesis) stated nonexposed animals had a mean ALP approximately twice the value reported here (208 ± 15.0), however the value fell within our 95% reference interval.[16] The difference in the mean might be due to methodology, because ALP was assayed in the previous study by using β-nitrophenyl phosphate rather than *p*-nitrophenyl phosphate as a reagent. Pine voles appear to have lower mean glucose than do prairie voles[20] and meadow voles[2,9,20] but higher glucose than do common voles (*Microtus arvalis*), which have been used for diabetes research (n = 7; ether anesthesia and cardiocentesis).[32]

Biochemical and hematologic reference values vary widely for laboratory mice (*Mus musculus*) and rats (*Rattus norvegicus*), depending in part on the specific strain, and as such, comparisons with data from pine voles are of limited value. However, as a general trend, pine voles in this study had a tendency to higher mean BUN, higher mean ALT, lower mean glucose, and lower mean phosphorous than published values for 2 other commonly used laboratory rodents (Crl:CD/SD rat and CD1/ICR mouse) within the same suborder Myomorpha (superfamily Muroidea) for samples collected by cardiocentesis.[4,6]

Examination of the histograms showed that the observed values of the BUN:creatinine ratio were plotted as bimodal, whereas the albumin:globulin ratio was normally distributed. This difference is likely because BUN and creatinine are independent parameters and as such alterations in either will shift the ratio away from 1. Alternatively, albumin and globulin have a direct physiologic relationship to each other in maintaining normal blood osmolality and, as such, a change in one often results in a change in the other.

The literature on the hematology of voles is more extensive than is that on their biochemistry. In published articles and textbook chapters (the latter sometimes lacking primary references), common voles (*Microtus arvalis*; n = 5 to 11; ether anesthesia and retroorbital plexus venipuncture),[10,11] field voles (*Microtus californicus*),[12] the tundra vole (*Microtus oeconomus operarius*),[8] and the meadow vole (previously the Eastern meadow mouse, *Microtus pennsylvanicus tananaensis*; n = 21; isoflurane anesthesia and nonlethal variable jugular, carotid, or cardiac puncture)[8,9,18] established similar hematologic values to those in our study for pine voles. These various species of voles possessed lower MCV and higher RBC count than most reported laboratory mice and rat strains,[5,7,23,24] some wild mouse species (that is, *Mastomys natalensis*), the Mongolian gerbil (*Meriones unguiculatus*),[22] and the Syrian hamster (*Mesocricetus auratus*).[25] The total oxygen-carrying capacity may be maintained between species, given that the factor of MCV and RBC would tend to be consistent if MCV decreases when RBC increases. This notion is supported by the finding that hemoglobin and MCHC are generally similar among all of these species despite as large as 2-fold differences in MCV.

Linear regression showed a significant weak positive correlation between age and hemoglobin, hematocrit and MCV and a weak negative correlation between age and MCHC. The findings suggest voles are similar to other species in which neonates and young juveniles have a mild transient macrocytic hypochromic anemia and are presumably iron-deficient relative to their adult counterparts. Evaluation of circulating iron levels and marrow stainable iron would be helpful to further evaluate this possibility but is likely unnecessary, because this species is used predominantly for reproductive behavior and endocrinology research involving mature adults.

A minority of our hematology samples were evaluated on analyzer B. However, given that 36% of the platelet measurements from hematology analyzer A were higher than the highest platelet value measured from hematology analyzer B, a true difference in their calculation of pine vole platelet numbers is probable. These 2 flow cytometer hematology analyzers use different methodologies that likely account for the observed difference in platelet reference intervals. The flow cytometer analyzer B uses traditional electrical impedance to measure platelets, whereas analyzer A uses routine and amplified 2-angle (low- and high-angle) laser scatter signals—this methodology has the advantage of better differentiation of small erythrocytes, RBC fragments, and RBC ghosts from platelets.[26] Hematology analyzer B has previously been reported to place a lower threshold for RBC volume when differentiating large platelets from small erythrocytes,[17] a factor that also might contribute to our findings. Alteration of the software on the analyzers to specifically accommodate pine voles might yield different results.

Normal plasma chemistry ranges have not previously been published, few normal hematologic findings are published for any species of voles, and none of the published data were calculated by using the recommended method of rank percentile analysis. Our study did not detect significant differences between male and female voles for any analyte. Except for platelet counts, significant differences were not found between hematologic analyses performed on the two different automated hematology analyzers. Relative to adults, younger animals have lower hematocrits, higher MCV, and lower MCHC, as is common for many mammalian species. The values reported in this study provide a useful resource for gauging the health status of pine voles maintained under similar environmental conditions, with blood collected by using similar techniques and analyzed on these hematology analyzers and this biochemistry machine.

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