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Normal Organ Weights, Serum Chemistry, Hematology, and Cecal and Nasopharyngeal Bacterial Cultures in the Gray Short-Tailed Opossum (*Monodelphis domestica*)

Kristin D Evans,[1,*] Terry A Hewett,[1] Cindy J Clayton,[3] Leah A Krubitzer,[3] and Stephen M Griffey[2]

Gray short-tailed opossums (*Monodelphis domestica*) currently are used in genetic, developmental, oncology, and neurologic research. Little is known about their natural flora or potential for pathogenic infectious disease. The present study aims to improve existing comparative normal blood and organ weight values available to researchers and to describe flora of clinically normal *M. domestica* to obtain an understanding of potential pathogenic flora in clinically abnormal animals. For evaluation of serum hematology and serum chemistry, clinically normal animals were assigned to 1 of 6 groups stratified by age (younger than 1 y, 1 to 2 y, and 2 to 3 y) and sex. Hemoglobin and phosphorus levels were higher in male than female opossums, whereas monocyte and eosinophil counts were greater in females than males. Hemoglobin concentration decreased with increasing age. The youngest group had significantly higher levels of serum alkaline phosphatase and lower serum protein levels compared with older age groups. Liver and kidney weights of adult animals (1 to 3 y) were greater in female than male opossums. The predominant nasopharyngeal flora in 20 clinically normal animals from the 2- to 3-y-old group were *Streptococcus viridans*, *Escherichia coli*, and coagulase-negative *Staphylococcus* spp.; predominant cecal organisms were *Escherichia coli* and *Citrobacter* spp. The availability of reference hematologic values and flora for *Monodelphis domestica* will aid researchers in comparisons and analysis of experimental data and in diagnosis and evaluation of potential pathogens in clinically ill animals.

The gray short-tailed opossum (*Monodelphis domestica*) was the first marsupial species to be produced in large numbers in captivity for use in experimental research.[14] These opossums are a prominent animal model currently used in genetic, developmental, oncology, and neurologic research. Little is known about their natural flora or potential for pathogenic infectious disease in a laboratory-reared environment. Limited studies have shown the gray short-tailed opossum is generally hardy, clean, and disease resistant.[2] However, a detailed retrospective pathology study showed that the greatest concentration of lesions seen in clinically ill animals lie within the digestive system. In addition, many of these lesions may be indicative of viral and bacterial pathogenesis due to unknown causative agents.[4] Characterization of bacterial milieu located within the gastrointestinal system of clinically normal laboratory-reared gray short-tailed opossums would contribute to our understanding of potential pathogenic bacteria isolated from clinically abnormal animals.

The establishment of the gray short-tailed opossum as a model for diet-induced hypercholesterolemia[9] and melanoma[15] and the discovery of its lack of mixed lymphocyte reaction[11] led to increased use of this animal as a model for human disease and developmental immunology research. Although comparative normal blood values for laboratory reared gray short-tailed opossum have been evaluated,[1,13] the methodology and equipment used in obtaining these parameters has improved vastly since their initial publication. The increased use of the gray short-tailed opossum as a model for human neurology and immunologic disease studies necessitates the need for updated comparative normal blood values.

The present study was undertaken in to describe flora of the clinically normal laboratory-reared gray short-tailed opossum to obtain a better understanding of potential pathogens and to improve existing comparative normal blood and organ weight values available to researchers.

Materials and Methods

Animals. The gray short-tailed opossum colony was established from breeding pairs obtained from Southwest Foundation for Biomedical Research (San Antonio, TX). All animal protocols were approved by the University of California–Davis Institutional Animal Care and Use Committee. The breeding colony is maintained as outbred on a rotational breeding scheme with addition of new stock approximately every 2 to 3 y for genetic diversity. The gray short-tailed opossum used in this study were maintained according to accepted animal care and use standards. Animals were either pair-housed (animals younger than 1 y) or singly housed (all other animals) in polycarbonate rodent cages fitted with stainless-steel wire lids and containing wood-pulp bedding (Carefresh, Absorption Corporation, Ferndale, WA) changed weekly. Animals were provided plastic cups

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[1]Center for Laboratory Animal Science and [2]Comparative Pathology Laboratory, School of Veterinary Medicine and [3]Department of Psychology, University of California Davis, Davis, California.
[*]Corresponding author. Email: kevans@ucdavis.edu

to nest in for environmental enrichment. Feeding consisted of 1 of 2 diets—National Complete Fox Reproduction Pellets (Milk Specialties, New Holstein, WI) or Purina Cat Chow (Nestle Purina, St Louis, MO)—fed ad libitum. At the time of sampling, all animals were in a fed state by using the Fox Reproduction diet. Animals were maintained under fluorescent lighting on a 14:10-h light:dark schedule at temperatures of 68 to 72 °F (20.0 to 22.2 ºC) and ambient humidity.

Assignment of animal groups. Clinically normal animals were assigned to 1 of 6 groups on the basis of age (younger than 1 y, $n = 16$; 1 to 2 y, $n = 11$; and 2 to 3 y, $n = 19$) and sex (male, $n = 26$; and female $n = 20$) for hematology and serum chemistry analysis. Opossum assigned to the 3 groups (younger than 1 y, 1 to 2 y, 2 to 3 y) ranged in age from 89 to 109 d, 435 to 611 d, and 779 to 1061 d, respectively, at the time of sampling. Nasopharynx and cecal cultures were collected from 20 clinically healthy 2- to 3-y-old animals. Because of the inherent variability in the organ weight of growing animals in addition to sexual immaturity, organ weights were measured in adult (1 y or older; $n = 29$) animals only.

Sample collection. At the time of sampling, animals were euthanized by CO_2 narcosis, and intracardiac blood samples were obtained by using 27-gauge needles and 3-mL syringes. Approximately 0.2 mL blood was placed into vacuum phlebotomy tubes containing powdered disodium EDTA, and 0.3 to 0.5 mL was placed into a serum separator collection tube (BD Biosciences, Franklin Lakes, NJ), allowed to clot, and centrifuged for 10 min at 4,000 × *g* (Spectrafuge 24D, Labnet International, Edison, NJ). Serum was recovered and analyzed immediately or stored for no longer than 12 h at 4 °C until analysis. Aseptic technique was used to obtain bacterial samples from the nasopharynx of 2- to 3-y-old animals and cultured on blood agar (Hardy Diagnostics, Santa Maria, CA). The same technique was used to obtain bacterial samples from the cecum of 2- to 3-y-old animals, and samples were cultured on MacConkey agar (Hardy Diagnostics). Organ weights were obtained for liver, spleen, left and right kidney, ovaries, testes, heart, and left and right adrenals of 1- to 2-y-old and 2- to 3-y-old animals by using a microscale (Explorer, Ohaus, Switzerland).

Bacterial analyses. Nasopharynx samples were grown aerobically at 37 °C at 5% CO_2 for a total of 72 h on blood agar. During the course of initial growth, isolated colonies were identified by using a combination of triple-sugar–iron, catalase, oxidase, and API bacterial identification kits (BioMerieux, Hazelwood, MO). Cecal samples were grown aerobically at 37 °C at 0% CO_2 for a total of 72 h on MacConkey agar, and isolated colonies were identified by using the kits listed for the nasopharyngeal samples.

Hematologic and differential analyses. Leukocyte and erythrocyte counts were performed by using an automated hematology analyzer (Hemavet 850 FS, Drew Scientific, Waterbury, CT), using the 'other nonspecies-specific' threshold setting. Undiluted whole blood (20 µL) mixed with EDTA provided analyses of leukocytes ($\times 10^3/mm^3$), erythrocytes ($\times 10^3/mm^3$), hemoglobin (g/dL), mean corpuscular volume (fL), mean corpuscular hemoglobin (pg), mean corpuscular hemoglobin concentration (g/dL), and platelets ($\times 10^3/mm^3$). Leukocyte cell differentials were determined from Wright-stained blood smears.

Serum chemistry analyses. Serum chemistry values were obtained by using an automated chemistry analyzer (Cobas Integra 400 Plus, Roche Diagnostics, Rotkreuz, Switzerland). Just prior to analysis, calibration and quality-control procedures were performed. Calibration was performed using by commercially prepared standard material (calibrator for automated systems) prepared specifically for use under the operating parameters for the system (Cobas Integra 400 Plus, Roche Diagnostics). This multiconstituent calibrator was used to identify confidence limits of 95% ± 2 SD to evaluate the quality of calibration. Approximately 100 to 150 µL serum was needed to obtain analyses for individual chemistry values for calcium, phosphorus, total protein, albumin, glucose, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase.

Table 1. Body and organ weights (mean ± SEM) in adult *Monodelphis domestica*

	Male ($n = 14$)	Female ($n = 16$)	P
Body weight	133.227 ± 3.350	92.367 ± 3.350	< 0.0001[a]
Liver	3.617 ± 0.160	4.395 ± 0.160	0.0020[a]
Spleen	0.452 ± 0.081	0.532 ± 0.081	0.4702
Left kidney	0.448 ± 0.011	0.504 ± 0.011	0.0012[a]
Right kidney	0.449 ± 0.011	0.510 ± 0.011	0.0008[a]
Left gonad	0.373 ± 0.018	0.018 ± 0.018	not done
Right gonad	0.382 ± 0.025	0.011 ± 0.025	not done
Heart	0.657 ± 0.048	0.650 ± 0.048	0.9277
Left adrenal	0.012 ± 0.001	0.011 ± 0.001	0.9777
Right adrenal	0.009 ± 0.005	0.008 ± 0.005	0.3222

Body weight is given in grams; the remaining parameters are given in %body weight
[a]Values are significantly different ($P < 0.05$) between male and female opossums

Statistical analyses. Statistical analyses were performed by using Statistical Analysis Software (version 9, SAS Institute, Cary, NC). Hematology, serum chemistry, and organ weight (as a percentage of body weight) were analyzed by using least-squares ANOVA, with sex and age treated as fixed effects (PROC GLM function). Comparisons were made between sexes, between ages, and for sex×age interaction. Statistical significance was defined as a P value of less than 0.05. Standard reference intervals were determined by using calculated means and 1 standard deviation (PROC MEANS function) for each measured blood parameter either as a pooled sample or stratified by age or sex if significantly affected by these variables as shown by ANOVA. Actual reference ranges represent 5% to 95% identity of normal population (mean ± 2 SD)

Results

Organ weights. Organ weights (as a percentage of body weight) of gray short-tailed opossums showed significant ($P < 0.05$) differences between sexes for overall body weight, liver, and left and right kidneys (Table 1). Males weighed 31% more than did females, whose liver (18%) and kidney (11%) weights were greater than those of males. There were no significant age effects or age×sex interactions noted for either organ weights or body weights within the age groups evaluated. Body weights and organ weights were not evaluated for animals younger than 1 y, because they are still in the growth phase.

Hematology and serum biochemistry. Hematology values affected by sex included hemoglobin, monocyte differential percentage, and eosinophil differential percentage (Table 2). Hemoglobin concentration was 8% greater in males than females. Erythrocyte number, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration did not differ between sexes, whereas monocyte and eosinophil percentages were greater in females (36% and 67%, respectively) than males. Serum biochemistry

Table 2. Hematologic and serum biochemistry values (mean ± SEM) of *Monodelphis domestica* by sex

	Male (n = 26)	Female (n = 20)	P
WBC ($\times 10^3/mm^3$)	16.84 ± 1.50	13.54 ± 1.50	0.1915
RBC ($\times 10^3/mm^3$)	6.80 ± 0.13	6.43 ± 0.17	0.0881
Hemoglobin (g/dL)	13.6 ± 0.3	12.6 ± 0.4	0.0455[a]
Hematocrit (%)	35.9 ± 0.8	34.0 ± 1.1	0.1665
Mean corpuscular volume (fL)	53.0 ± 1.0	53.0 ± 1.3	0.9813
Mean corpuscular hemoglobin (pg)	20.0 ± 0.3	19.7 ± 0.4	0.5899
Mean corpuscular hemoglobin concentration (g/dL)	37.8 ± 0.5	37.2 ± 0.6	0.4616
Platelets ($\times 10^3/mm^3$)	312 ± 23	334 ± 30	0.5678
Neutrophils (%)	28 ± 2	23 ± 3	0.2187
Lymphocytes (%)	52 ± 3	48 ± 4	0.4214
Monocytes (%)	14 ± 1	19 ± 2	0.0196[a]
Eosinophils (%)	6 ± 1	10 ± 1	0.0485[a]
Basophils (%)	0.14 ± 0.13	0.52 ± 0.17	0.0894
Blood urea nitrogen (mg/dL)	55.0 ± 1.4	53.9 ± 1.8	0.6057
Creatinine (mg/dL)	0.17 ± 0.01	0.17 ± 0.01	0.7935
Total bilirubin (mg/dL)	0.20 ± 0.02	0.17 ± 0.02	0.4049
Alkaline phosphatase (U/L)	99 ± 7	110 ± 10	0.3898
Alanine aminotransferase (U/L)	134 ± 11	132 ± 15	0.9192
Aspartate aminotransferase (U/L)	85 ± 12	84 ± 16	0.9313
Calcium (mg/dL)	10.3 ± 0.1	10.2 ± 0.2	0.7617
Phosphorus (mg/dL)	7.2 ± 0.2	6.0 ± 0.3	0.0050[a]
Total protein (g/dL)	6.4 ± 0.1	6.5 ± 0.1	0.4432
Albumin (g/dL)	3.7 ± 0.1	3.7 ± 0.1	0.8078
Glucose (mg/dL)	129 ± 18	122 ± 24	0.8234

[a]Values are significantly different ($P < 0.05$) between male and female opossums.

values were largely unaffected by sex. However, phosphorus levels in male opossums were 20% higher than those in female opossums.

Hematology values affected by age included hemoglobin and mean corpuscular hemoglobin concentration (Table 3). A graded decrease in hemoglobin concentration with age was noted between the 2 youngest age groups. Nonadult (younger than 1 y) animals exhibited 11% more hemoglobin compared with 2- to 3-y-olds. In addition mean corpuscular hemoglobin concentration (7%) was greater in the youngest group compared with 2- to 3-y-old animals. Erythrocyte cell numbers, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin were not different between ages. Serum biochemistry values affected by age included alkaline phosphatase and total protein. Alkaline phosphatase levels (43% to 76%) were highest in animals younger than1 y compared with both older age groups. Total protein levels in the youngest opossums (6%) were significantly ($P < 0.05$) lower than those in both older age groups. Albumin levels were unaffected by age, and globulin levels were not evaluated.

No statistically significant sex×age interactions noted. Therefore, we generated a table containing normal values hematology and serum biochemistry parameters in *Monodelphis domestica* (Table 4). that encompassed the sex- and age-associated differences that emerged.

Normal nasopharyngeal and cecal flora. The predominant nasopharyngeal bacterial flora was *Streptococcus viridians*, which was isolated from 50% of all opossums surveyed. *Escherichia coli* (35%) and coagulase-negative *Staphylococcus* (25%) also were obtained from surveyed animals. Organisms cultured less frequently included *Bacillus* spp. (15%), *Enterococcus* spp. (15%), group D non*Enterococcus* (10%), *Lactobacillus* spp. (10%), and *Staphylococcus lentus* (5%). Approximately 25% of all animals surveyed exhibited no growth of nasopharyngeal cultures after 72 h. The predominant cecal bacterial flora was *Escherichia coli* (95%). One animal surveyed yielded *Citrobacter* spp. (5%). In contrast to cultures from the nasopharynx, cecal cultures from only 5% of animals surveyed showed no growth after 72 h.

Discussion

Body weight data reported in this study concur with previous results from gray short-tailed opossums.[7] However, the current study reports differences between males and females with respect to liver and kidney weights (on a percentage body weight basis) that is relevant to evaluation of this animal as a model in any future toxicology studies.[10] *Monodelphis* animals reach sexual maturity at around 7 mo, and the average lifespan in captivity is 3 y, with females generally surviving longer than males.[6,7] Therefore, the most common age range at which animals are used on study is between 1 and 3 y of age.

The hematologic differences between sex and age group in the current study were similar to those seen in previously published hematology profiles from gray short-tailed opossums. The present study identified larger mean hemoglobin values in males compared with females, whereas all other erythrocyte parameters were unaffected by sex. Previous studies evaluating primarily diet-related effects in gray short-tailed opossums revealed sex-related differences in hemoglobin and erythrocyte number.[13] A later study conducted to evaluate specific age- and sex-related differences in hematologic values noted differences between sexes for erythrocyte number, mean corpuscular volume, and mean corpuscular hemoglobin concentration, with the difference in hemoglobin approaching significance.[1] Other

Table 3. Hematologic and serum biochemistry values (mean ± SEM) of *Monodelphis domestica* by age

	< 1 y (*n* = 16)	1–2 y (*n* = 11)	2–3 y (*n* = 19)	*P*
WBC ($\times 10^3/mm^3$)	14.11 (± 2.12)	16.25 (± 2.48)	15.23 (± 1.81)	0.8042
RBC ($\times 10^3/mm^3$)	6.74 (± 0.18)	6.71 (± 0.21)	6.39 (± 0.16)	0.2800
Hemoglobin (g/dL)	13.7 (± 0.4)[b]	13.2 (± 0.5)[b]	12.3 (± 0.3)[a]	0.0384
Hematocrit (%)	36.0 (± 1.2)	34.6 (± 1.4)	34.4 (± 1.0)	0.5518
Mean corpuscular volume (fL)	53.5 (± 1.4)	51.5 (± 1.6)	54.1 (± 1.2)	0.4450
Mean corpuscular hemoglobin (pg)	20.4 (± 0.5)	19.7 (± 0.5)	19.4 (± 0.4)	0.2296
Mean corpuscular hemoglobin concentration (g/dL)	38.3 (± 0.7)[b]	38.3 (± 0.8)[b]	35.9 (± 0.6)[a]	0.0177
Platelets ($\times 10^3/mm^3$)	317 (± 32)	327 (± 38)	326 (± 28)	0.9745
Neutrophils (%)	26 (± 3)	21 (± 4)	31 (± 3)	0.1558
Lymphocytes (%)	51 (± 4)	52 (± 5)	47 (± 4)	0.6186
Monocytes (%)	16 (± 2)	17 (± 2)	15 (± 2)	0.7469
Eosinophils (%)	8 (± 1)	9 (± 2)	7 (± 1)	0.6709
Basophils (%)	0.25 (± 0.18)	0.5 (± 0.22)	0.24 (± 0.16)	0.5902
Blood urea nitrogen (mg/dL)	53.8 (± 1.9)	57.8 (± 2.2)	51.8 (± 1.6)	0.1068
Creatinine (mg/dL)	0.19 (± 0.01)	0.17 (± 0.02)	0.15 (± 0.01)	0.1027
Total bilirubin (mg/dL)	0.19 (± 0.03)	0.20 (± 0.03)	0.16 (± 0.02)	0.6410
Alkaline phosphatase (U/L)	139 (± 10)[b]	97 (± 12)[a]	79 (± 9)[a]	0.0003
Alanine aminotransferase (U/L)	152 (± 16)	104 (± 19)	142 (± 14)	0.1462
Aspartate aminotransferase (U/L)	96 (± 17)	65 (± 19)	92 (± 14)	0.4276
Calcium (mg/dL)	10.3 (± 0.2)	10.4 (± 0.2)	10.2 (± 0.2)	0.8390
Phosphorus (mg/dL)	6.8 (± 0.3)	6.5 (± 0.4)	6.5 (± 0.3)	0.7957
Total protein (g/dL)	6.2 (± 0.1)[a]	6.6 (± 0.1)[b]	6.6 (± 0.1)[b]	0.0403
Albumin (g/dL)	3.8 (± 0.1)	3.7 (± 0.1)	3.5 (± 0.1)	0.0814
Glucose (mg/dL)	175 (± 26)	106 (± 30)	96 (± 22)	0.0653

Different superscripts within a given measured parameter indicate values that are significantly ($P < 0.05$) different

previously obtained values[1,13] included greater hemoglobin values in males compared with females similar to the present study. However, the previously published mean hemoglobin values[1,13] were significantly different and contained larger standard deviations than those observed in the present study. In particular, previously reported mean values for hemoglobin were 14.42 and 15.37 g/dL for males and 14.13 and 14.42 g/dL for females.[1,13] These values are strikingly different from the mean values of 13.6 g/dL for male and 12.6 g/dL for female opossums that we obtained in the present study. In addition, the present study revealed no difference in mean values for any of the other erythrocyte parameters evaluated in gray short-tailed opossums in response to sex-associated differences, which are well known among other species. Studies in humans show varying changes in response to age and sex, with several studies[5,8] noting increased hemoglobin in males compared with females and no change in mean corpuscular hemoglobin concentration, similar to results from the current study. This difference often is attributed to the lower oxygen affinity of female hemoglobin.[5] In the current study, hemoglobin was measured by using the spectrophotometric cyanomethemoglobin method. Consequently hemoglobin measurement was highly reliant on the binding affinity of hemoglobin to the cyano compound, which binding is dependent on the oxygen-binding affinity of hemoglobin.

Differences in monocyte and eosinophil differential values emerged in the present but not previous studies. However, female opossums previously had greater (but not significantly so) monocyte and eosinophil numbers.[13] Because the previous study evaluated diet-associated differences in hematology data, differences in monocyte and eosinophil numbers might have been attributed to diet thus obscuring any true sex-associated differences. In addition, monocyte and eosinophil numbers are known to increase during pregnancy and parturition and in response to progesterone.[12] Although the opossums used in the present study were not breeding currently, the eosinophil and monocyte responses in this study could be attributed to progesterone levels in females and were significant due to the updated methods of blood analysis used, which therefore had increased sensitivity to detect sex-associated changes in these values.

In addition to sex-related differences in hemoglobin, age also appeared to alter this value. In the present study, opossums younger than 1 y had significantly higher hemoglobin and mean corpuscular hemoglobin than did older animals. These effects of age were not noted in prior studies.[1] In addition, previous studies[1,13] reported sex×age interaction, which was not seen in the current study. Potential reasons for the differences seen between this and previous studies may relate to the advances in diagnostic machinery that have occurred between the mid1980s and 2008. The hematology values collected previously[1,13] were analyzed by using an automatic diluting cell counter. The data collected in our study were obtained by using a whole-blood veterinary-grade cell counter with species-specific counting capability. The advantages of the current system are that the current system runs off whole-blood samples instead of diluted samples, is designed specifically for veterinary use with a wider range of sampling for given hematology parameters, can establish hematology results with very small samples (20 µL), and uses species-specific modules to account for differences in cell size between species.

The serum biochemistry results in the current study displayed similar trends to those previously reported, with some distinct differences. Between sexes, serum phosphorus levels in the current study were greater in male opossums than females.

Table 4. Recommended hematology and serum biochemistry reference values for *Monodelphis domestica* ,accounting for age- and sex-associated effects

		Mean	Reference range	No. of animals
WBC ($\times 10^3/mm^3$)	Overall	16.01	12.25–19.77	46
RBC ($\times 10^3/mm^3$)	Overall	6.56	6.22–6.90	46
Hemoglobin (g/dL)	Overall	13.0	12.2–13.8	46
	Male	13.6	13.0–14.2	26
	Female	12.6	11.8–13.4	20
	0–2 y	13.2	12.2–14.2	27
	2–3 y	12.3	11.7–12.9	19
Hematocrit (%)	Overall	34.8	32.6–37.0	46
Mean corpuscular volume (fL)	Overall	53.2	50.6–55.8	46
Mean corpuscular hemoglobin (pg)	Overall	19.9	19.1–20.7	46
Mean corpuscular hemoglobin concentration (g/dL)	Overall	37.5	36.3–38.7	46
	0–2 y	38.5	36.9–40.2	27
	2–3 y	36	34.8–37.2	19
Platelets ($\times 10^3/mm^3$)	Overall	321	261–381	46
Neutrophils (%)	Overall	27	21–33	46
Lymphocytes (%)	Overall	51	43–59	46
Monocytes (%)	Overall	15	11–19	46
	Male	14	12–16	26
	Female	19	15–23	20
Eosinophils (%)	Overall	7	5–9	46
	Male	6	4–8	26
	Female	10	8–12	20
Basophils (%)	Overall	0.25	0–0.59	46
Blood urea nitrogen (mg/dL)	Overall	54.5	50.9–58.1	46
Creatinine (mg/dL)	Overall	0.17	0.12–0.22	46
Total bilirubin (mg/dL)	Overall	0.18	0.09–0.27	46
Alkaline phosphatase (U/L)	Overall	101	60–142	46
	< 1 y	139	119–159	16
	1–3 y	86	64–108	30
Alanine aminotransferase (U/L)	Overall	134	78–190	46
Aspartate aminotransferase (U/L)	Overall	86	29–145	46
Calcium (mg/dL)	Overall	10.2	9.6–10.8	46
Phosphorus (mg/dL)	Overall	6.7	5.4–8.0	46
	Male	7.2	6.8–7.8	26
	Female	6.0	5.4–6.6	20
Total protein (g/dL)	Overall	6.4	5.9–6.9	46
	< 1 y	6.2	6.0–6.4	16
	1–3 y	6.6	6.4–6.8	30
Albumin (g/dL)	Overall	3.6	3.2–4.0	46
Glucose (mg/dL)	Overall	129	69–189	46

This result was not readily seen in the age- and sex-related serum biochemistry values previously reported.[1] In addition, the current study saw no sex×age interaction with phosphorus levels, in contrast to previous studies. Previously reported data[1] included differences in calcium, aspartate aminotransferase, and alanine aminotransferase between different ages. The current data showed differences in only total protein and alkaline phosphatase values. As expected, alkaline phosphatase values were increased in animals younger than 1 y compared with 1- to 2-y and 2- to 3-y-old animals. This effect can be attributed to the increased extra hepatic enzyme activity seen in other mammal species in the growing phase of their life cycle.[3] Total protein values were lower in the youngest age group compared with both older groups. These effects were not noted in previous studies. Potential reasons for the differences in serum biochemistry values seen in the current study compared with past studies include differences in sampling times and grouping between ages analyzed and differences in analyzing equipment. Previous studies conducted looked at age differences every 6 mo for 3.5 y.[1] Although this frequency may reveal subtle changes in age-associated differences that occur at specific age points, using these data to develop a standard reference range becomes problematic because different values peak and change at different times throughout an animal's life cycle. The current study adopted a more physiologic approach to age stratification, choosing animals younger than y age to represent the time after puberty at 5 to 6 mo and onset of stable weight, 1- to 2-y-old opossums to represent the time of greatest reproductive ability, and 2- to 3-y-old animals to represent breeding senescence and increased age-related pathology.[4,7] Serum chemistry sam-

ple analysis, similar to hematology analysis, has shown great advances in the 20 y since the publication of previous serum biochemical data for gray short-tailed opossums. Previously reported serum biochemistry data were analyzed by using a system that required large test volumes of serum for accuracy and monochromatic analysis, which can be skewed by serum color.[1] The current study analyzed serum biochemistry data by using a newer system whose advantages include minimal test volume (approximately 150 µL serum needed for entire chemistry profile) and direct sample blanking and bichromatic analysis, minimizing serum color effects and providing greater specificity.

Bacterial culture analysis of the nasopharynx and cecum of clinically normal animals provided a picture of the normal bacterial flora associated with the gray short-tailed opossum that will be useful for comparison studies and evaluation of animals that are showing clinical signs of disease. In conclusion, the organ weights, bacterial culture data, and hematology and serum biochemistry results reported in this study provide a valuable reference for researchers using gray short-tailed opossums. The values for hematology, serum biochemistry, and organ weights provide more accurate estimates of means and ranges in healthy gray short-tailed opossum than do those previously reported.[1]

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