Hematologic and Biochemistry Reference Values for the Endangered Riparian Brush Rabbit (Sylvilagus Bachmani Riparius)

Authors: Black, Dorothy M., Gilardi, Kirsten V. K., Hamilton, Laurissa P., Williams, Elizabeth, Williams, Daniel F., et al.

Source: Journal of Wildlife Diseases, 45(2) : 491-496

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-45.2.491

SHORT COMMUNICATIONS

Journal of Wildlife Diseases, 45(2), 2009, pp. 491–496

Hematologic and Biochemistry Reference Values for the Endangered Riparian Brush Rabbit (*Sylvilagus Bachmani Riparius*)

Dorothy M. Black,[1,4,7] **Kirsten V. K. Gilardi,**[1] **Laurissa P. Hamilton,**[2] **Elizabeth Williams,**[2,5] **Daniel F. Williams,**[2,6] **Patrick A. Kelly,**[2] **and Ian Gardner**[3] [1] Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, California 95616, USA; [2] Endangered Species Recovery Program, California State University, Stanislaus, Turlock, California 95382, USA; [3] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California 95616, USA; [4] Current address: Matthew J Ryan Veterinary Hospital Emergency Critical Care, 3900 Delancey St., Philadelphia, Pennsylvania 19104-6010, USA; [5] Current address: Tennessee Department of Environment and Conservation, 1625 Hollywood Drive, Jackson, Tennessee 38305, USA; [6] Current address: Endangered Species Recovery Program, 3850 NW Walnut Ave., Redmond, Oregon 97756, USA; [7] Corresponding author (email: mdblack@vet.upenn.edu)

ABSTRACT: The objective of this study was to establish reference hematologic and biochemistry values for adult riparian brush rabbits (*Sylvilagus bachmani riparius*). Between February 2002 and December 2003, complete blood cell counts and serum biochemistry panels were done on blood samples collected for 252 healthy, adult riparian brush rabbits that were bred in captivity and released to the wild in the California, USA, San Joaquin Valley. Significant differences in mean values of some analytes were found among males, nonpregnant females, and pregnant females and among seasons. However, when reference values for each subgroup (sex, pregnancy status, and season) were compared with reference values for all riparian rabbits combined, differences were not sufficiently relevant clinically to warrant the adoption of separate subgroup-specific reference values. The reference ranges reported herein will be of value to veterinarians and wildlife biologists assessing the health of riparian brush rabbits and other wild *Sylvilagus* species.

Key words: Reference values, riparian brush rabbit, *Sylvilagus bachmani riparius*.

The riparian brush rabbit (*Sylvilagus bachmani riparius*) is a federally endangered species endemic to California's San Joaquin Valley. Agricultural practices, urban development, fire, and diversion of waterways have nearly eliminated the essential above-floodplain riparian habitat critical for *S. bachmani riparius* survival (Williams et al., 1999). As of 2000, the world population of riparian brush rabbits comprised two known small populations along the Stanislaus and San Joaquin rivers, with most riparian brush rabbits living in Caswell Memorial State Park, Ripon, California, USA (Williams et al., 1999). To aid species recovery, the US Fish and Wildlife Service and California State University Stanislaus' Endangered Species Recovery Program (ESRP) initiated the Riparian Brush Rabbit Controlled Propagation and Reintroduction Program (RBR program) in 2001, with the goal of establishing three self-sustaining populations outside of Caswell Memorial State Park (Williams et al., 1999). The RBR program presented a unique opportunity to obtain a substantial number of blood samples for establishing hematologic and biochemistry reference values for this endangered species. The objective of this study was to establish complete blood cell (CBC) count and serum biochemistry reference values for the riparian brush rabbit and to evaluate variation in the analytes by season, sex, and pregnancy status to determine whether it was important to establish reference values specific for these subgroups.

Blood samples were collected from 265 adult (>400 g) riparian brush rabbits during a 22-mo period (21 February 2002 to 17 December 2003). Study rabbits were trapped from multiacre natural vegetation cloth-netted enclosures established by the RBR program in Caswell Memorial state park. All rabbits included in this study were later released into the wild. Blood samples from 122 males, 105 nonpregnant females, and 25 pregnant females were analyzed. Samples were obtained in the spring (March to May; $n=1$), summer (June to August; $n=54$), fall (September to November; $n=148$), and winter (December to February; $n=49$). Rabbits were handled and samples were obtained according to the US Fish and Wildlife Service's *Controlled Propagation and Reintroduction Plan* (Williams et al., 2009). Rabbits were trapped in the morning using standard front-and-rear release wire-mesh traps baited with food (Tomahawk™, Tomahawk Live Trap, Tomahawk, Wisconsin, USA). Each rabbit was manually removed from the trap and anesthetized using isoflurane (IsoFlo™, Abbott Laboratories, North Chicago, Illinois, USA) delivered by mask. Rectal temperature, heart rate, and respiratory rate were recorded throughout the anesthetic period. The identification (ear tag and passive integrated transponder [PIT] tag) numbers, date of the physical examination, sex, and weight of each rabbit were recorded. Pregnancy status of females was determined by abdominal palpation. Abnormalities noted on the health form included, but were not limited to, rectal temperature, respiratory and cardiac abnormalities, wounds, palpable abdominal masses, and congenital defects. A maximum volume of 1.0 ml of blood was collected with 25-gauge needles and 1-ml syringes from the central auricular vein of each rabbit, and the sample was separated into a 500-μl Microtainer™ brand uncoated microtube and a microtube containing ethylenediaminetetraacetic acid (EDTA; Becton, Dickinson, & Co., Franklin Lakes, New Jersey, USA). The EDTA tubes were gently mixed and placed into a cooler. Uncoated microtubes were first allowed to clot at ambient temperatures (5–10 min) and were then placed into a cooler. Samples were transported to the Hematology and Clinical Chemistry diagnostic laboratories at the Veterinary Medical Teaching Hospital at the University of California, Davis, USA, within 6 hr of sampling and were processed within 24 hr of receipt.

Hematologic analysis of EDTA-anticoagulated whole-blood samples was performed with an ADVIA 120™ automated cell-counting machine (The Jackson Laboratory, Bar Harbor, Maine, USA). White blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell diameter width, platelet count, mean platelet volume, neutrophil count, lymphocyte count, monocyte count, eosinophil count, and basophil count were measured on each sample with this instrument. Serum samples were evaluated with a Hitachi 717™ automated chemistry analyzer (Roche Diagnostics, Inc., Indianapolis, Indiana, USA). Variables measured included total carbon dioxide, calcium, phosphorus, creatinine, serum urea nitrogen, glucose, total protein, albumin, globulin, alanine amino transferase, aspartate amino transferase, alkaline phosphatase, total bilirubin, cholesterol, and gamma glutamyl transferase. Before statistical analysis of the data, each rabbit record was reviewed, and the rabbit was classified as healthy or not healthy at the time of blood collection. Rabbits with two or more abnormalities or a single severe abnormality were considered not healthy ($n=13$), and their blood samples were excluded from further analysis.

Data were transferred into statistical analysis programs (SPSS 12.0™, SPSS Inc., Chicago, Illinois, USA, and MedCalc™ 9.0.1.1, MedCalc Software, Mariakerke, Belgium) and the mean, median, range, kurtosis, and skewness for each hematologic and serum biochemistry variable were evaluated. Stem leaf and box plots were also created. Outliers on these plots were identified, their record reviewed, and errors were corrected where necessary. Reference values for each variable were derived by calculating the central 95% interval (between the 2.5 and 97.5 percentiles) according to the International Federation of Clinical Chemistry (IFCC) protocol using a nonparametric procedure (Solberg, 1983; Lumsden, 1998). A 90% confidence interval was calculated for each percentile. A Kruskal-Wallis one-way analysis of variance for *k*-independent samples was used to evaluate differences in blood variables by sex, pregnancy status and season. $P \leq 0.05$ was considered statistically significant. The Dunn's post hoc test for unequal sample sizes was used to identify subgroup relationships. Finally, independent reference values were determined for subgroups.

Reference values based on the central 95% interval were calculated for 239 CBCs and 235 serum biochemistry panels (Table 1). Significant differences by sex and season were detected for several analytes (data not shown). Variation among groups may reflect difference in sample size, intraspecies variability, secondary response to stress, hormonal oscillation, disparity in energy expenditure with season, confounding factors, or unknown disease processes. Other studies of wild rabbits (*Sylvilagus floridanus*) demonstrated similar sex differences as were found in our study of riparian brush rabbits (Jacobson and McGinnes, 1978; Lepitzki and Woolf, 1991). Hematologic and biochemistry changes during pregnancy and lactation have also been reported (Bortolotti et al., 1989; Wells et al., 1999). Although other studies have defined seasonal periods differently, they reported similar trends of decreasing albumin and total protein in spring/summer months, and increasing packed cell volume/hematocrit in winter months (Jacobson and McGinnes, 1978; Lepitzki and Woolf, 1991). However, much of the variation we detected among sexes and seasons in our riparian brush rabbits is accounted for within the reference values and 95% intervals calculated for the species as a whole (Table 2). Hence, we believe that reference values and ranges established for riparian brush rabbits regardless of gender and season will be adequate for clinical decision-making.

In comparison to other species, there is, overall, a great deal of similarity. The previous reference values of the European rabbit (*Oryctolagus cuniculus*) encompassed most of the riparian rabbit reference values. However, there were several biochemistry values that trended higher for the riparian rabbits, such as alkaline phosphatase, serum alanine aminotransferase, phosphorus, and calcium (e.g., alkaline phosphatase reference values: 4–16 IU/l for European rabbits and 44–402 IU/l for riparian rabbits). Further comparisons and conclusions between studies and reference values, however, are difficult to draw because of variability in sample sizes, sampling methods, subject manipulation (anesthetized or shot versus awake), blood analyzers used, and other subject and equipment differences.

In summary, hematologic and biochemistry reference values can be useful in the evaluation of general and organ-specific health of animals. Reference values vary among species, seasons, sexes, and restraint/sampling procedures (Jacobson and McGinnes, 1978; Jacobson et al., 1978; Lepitzki and Woolf, 1991). Establishing reference ranges for wildlife—and in particular, for endangered species—can be difficult because of limited access to sufficient numbers of individuals for sampling. Therefore, it is often necessary for veterinarians and biologists to interpret hematologic data for their study species by extrapolating from published reference ranges for other related species or taxa. Indeed, the RBR program initially used published reference ranges for other *Sylvilagus* species and for European rabbits (*Oryctolagus cuniculus*) to interpret laboratory data for riparian brush rabbits. The results of this study will support the health assessment of riparian brush rabbits and potentially other *Sylvilagus* species.

The authors wish to thank the staff of the California State University (CSU) Stanislaus Endangered Species Recovery Program for their many hours of work in

TABLE 1. Hematologic and biochemistry reference values for adult riparian brush rabbits (*Sylvilagus bachmani riparius*). Reference values are the 2.5 and 97.5 percentiles and 90% confidence intervals (CI) estimated nonparametrically according to the IFCC[a] protocol; n=235 to 239 for each variable.

	Reference values			
Variable (units)[b]	2.5 percentile (lower reference limit)	90% CI	97.5 percentile (upper reference limit)	90% CI
WBC ($\times 10^9$/l)	3.3	2.4–3.6	12.8	11.3–15.3
RBC ($\times 10^{12}$/l)	4.6	4.5–4.8	7.3	7.1–7.4
HGB (g/dl)	9.6	9.4–9.8	14.6	14.4–14.9
HCT (%)	29.7	29.0–30.5	45.8	45.0–46.5
MCV (fl)	56.4	55.7–57.1	71.0	70.3–71.7
MCH (pg)	18.1	17.7–18.6	22.4	22.3–23.0
MCHC (g/dl)	30.0	29.5–30.4	34.2	33.8–34.5
RDW (%)	11.6	11.3–11.8	16.2	15.3–16.6
Platelet ($\times 10^9$/l)	138	85–190	1,247	1,194–1,363
MPV (fl)	5.2	5.0–5.3	9.6	9.3–9.9
Neutrophils ($\times 10^9$/l)	0.5	0.3–0.6	3.6	3.1–4.0
Lymphocytes ($\times 10^9$/l)	1.8	1.2–2.1	9.6	8.9–12.1
Monocytes ($\times 10^9$/l)	0.04	0.03–0.06	0.65	0.55–0.83
Eosinophils ($\times 10^9$/l)	0.01	0.00–0.02	0.28	0.25–0.31
Basophils ($\times 10^9$/l)	0.04	0.04–0.06	0.31	0.27–0.39
Total CO_2 (mmol/l)	11	10–12	25	24–26
Calcium (mmol/l)	2.3	2.03–2.48	3.4	3.35–3.5
Phosphorus (mmol/l)	1.3	1.22–1.49	3.2	3.07–3.33
Creatinine (μmol/l)	17.7	8.84–26.52	70.7	61.88–79.56
Serum urea nitrogen (mmol/l)	2.9	2.50–3.21	13.2	10.35–15.35
Glucose (mmol/l)	6.3	5.94–6.66	19.7	18.59–20.76
Total Protein (g/l)	52	51–53	73	72–74
Albumin (g/l)	32	31–33	48	47–49
Globulin (g/l)	15	14–15	32	31–33
A/G Ratio	1.0	0.92–1.09	2.8	2.70–2.87
Alanine aminotransferase (IU/l)	34	32–37	146	136–156
Aspartate aminotransferase (IU/l)	7.5	7–8	39.7	36.7–43.0
Alkaline phosphatase (IU/l)	44	39–55	402	364–487
Total bilirubin (μmol /l)	1.7	1.7–1.7	6.8	6.8–6.8
Cholesterol (mmol /l)	0.2	0.08–0.26	2.5	2.28–3.13
γ-Glutamyl-transferase (IU/L)	6	4–7	34	33–36

[a] IFCC = International Federation of Clinical Chemistry.

[b] WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell diameter width; MPV = mean platelet volume; CO_2 = carbon dioxide; A/G Ratio = albumin/globulin ratio.

trapping and handling riparian brush rabbits and to M. Christopher (School of Veterinary Medicine, University of California, Davis) for assistance with data analysis. Rabbits were handled under US Fish and Wildlife Service Permit WILLDF, and a Memorandum of Understanding between CSU Stanislaus and the California Department of Fish and Game. Animal care and use protocols were approved by CSU Stanislaus. We would also like to acknowledge funding and logistical support of this research and riparian brush rabbit recovery by the US Bureau of Reclamation, the Central Valley Project Conservation Program and Habitat Restoration Program, the US Fish and Wildlife Service, the California Department of Fish and Game, the California Department of Water Resources, the California Department of Parks and Recreation, and Susan Dell'Osso (Cambay Group).

TABLE 2. Reference ranges for adult riparian brush rabbits (RBR[a]; *Sylvilagus bachmani riparius*), including by sex, pregnancy status, and season (defined as 2.5 to 97.5 percentiles, from Table 1).

Variable[b]	RBR (n=235–239)	Male (n=114–115)	Nonpregnant female (n=97–99)	Pregnant female (n=23–24)	Summer (n=48–50)	Fall (n=139–140)	Winter (n=46)
WBC ($\times 10^9$/l)	3.3 –12.8	2.9–11.5	3.5–13.8	1.8–11.1	3.9–12.0	3.4–12.8	2.4–11.2
RBC ($\times 10^9$/l)	4.6–7.3	4.6–7.3	5.1–7.3	4.6–6.3	4.5–6.9	4.7–7.1	5.4–7.7
HGB (g/dl)	9.6–14.6	9.7–14.6	10.6–15.3	9.2–12.6	10.1–14.2	9.5–14.1	11.1–15.3
HCT (%)	29.7–45.8	29.9–45.9	32.6–48.1	29.1–39.8	32.0–45.9	29.5–44.4	32.7–48.1
MCV (fl)	56.4–71.0	56.8–71.4	56.9–72.3	59.8–75.0	58.8–75.0	56.8–68.7	56.9–71.8
MCH (pg)	18.1–22.4	17.8–22.3	18.2–22.6	18.8–22.8	19.3–23.4	17.9–22.0	18.3–22.3
MCHC (g/dl)	30.0–34.2	30.0–33.9	30.0–34.5	29.5–33.7	30.0–33.7	30.1–34.4	30.0–33.9
RDW (%)	11.6–16.2	11.6–16.2	11.4–16.4	11.8–15.4	44.5–15.1	11.6–15.3	11.7–16.6
Platelet ($\times 10^9$/l)	138–1247	150–1265	124–1183	303–1313	189–1175	150–1291	161–999
MPV (fl)	5.2–9.6	5.3–9.5	5.4–9.9	5.4–11.4	4.5–9.4	5.7–10.4	5.5–9.3
Neutrophils($\times 10^9$/l)	0.5–3.6	0.6–3.8	0.5–3.6	0.2–2.6	0.5–3.8	0.6–3.3	0.5–3.3
Lymphocytes($\times 10^9$/l)	1.8–9.6	1.7–9.6	1.9–9.9	1.4–10.1	2.1–9.0	2.0–9.7	1.0–8.8
Monocytes ($\times 10^9$/l)	0.04–0.65	0.04–0.59	0.07–0.74	0.03–0.47	0.04–0.49	0.04–0.71	0.06–0.49
Eosinophils ($\times 10^9$/l)	0.01–0.28	0.01–0.25	0.01–0.28	0.03–0.40	0.00–0.28	0.02–0.26	0.02–0.28
Basophils ($\times 10^9$/l)	0.04–0.31	0.05–0.36	0.04–0.30	0.03–0.24	0.08–0.32	0.05–0.30	0.04–0.27
Total CO_2 (mmol/l)	11–25	11–25	10–25	11–23	11–24	11–23	10–27
Calcium (mmol/l)	2.3–3.4	2.7–3.4	2.3–3.4	2.1–3.3	2.3–3.3	2.4–3.4	2.5–3.5
Phosphorus (mmol/l)	1.3–3.2	1.3–3.0	1.5–3.3	0.9–3.3	1.3–3.3	1.4–3.2	1.4–3.2
Creatinine (μmol/l)	17.7 –70.7	17.7–70.7	17.7–70.7	8.8–61.9	26.5–61.9	17.7–61.9	26.5–88.4
SUN (mmol/l)	2.9–13.2	2.9–13.6	3.2–13.9	2.5–8.6	2.9–9.3	3.2–8.2	4.3–15.7
Glucose(mmol/L)	6.3–19.7	6.2–21.8	6.9–20.0	6.4–23.6	7.7–19.9	6.9–23.6	6.0–14.5
Total protein (g/l)	52–73	54–72	51–74	51–67	51–70	52–72	56–74
Albumin (g/l)	32–48	34–49	32–47	30–44	31–43	32–47	37–49
Globulin (g/l)	15–32	14–34	16–33	16–30	16–26	16–33	14–28
A/G ratio	1.0–2.8	1.0–2.9	1.1–2.7	1.0–2.1	1.2–2.4	1.4–3.5	1.1–2.9
ALT (IU/l)	34–146	40–164	43–149	30–93	37–101	37–123	58–165
AST (IU/l)	7–40	7–49	8–47	8–30	7–34	9–42	8–50
ALP (IU/l)	44–402	41–444	52–364	43–428	65–351	55–428	42–308
Total bilirubin (μmol/l)	1.7–6.8	1.7–6.8	1.7–6.8	1.7–6.8	1.7–6.8	1.7–6.8	1.7–6.8
Cholesterol (mmol/l)	0.2–2.5	0.2–2.3	0.6–2.6	0.1–1.1	0.2–2.0	0.2–2.6	0.4–2.3
GGT (IU/l)	6–34	10–36	9–34	6–25	9–28	8–35	18–37

[a] RBR = riparian brush rabbits.

[b] WBC=white blood cell count; RBC=red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell diameter width; MPV = mean platelet volume; CO_2 = carbon dioxide; SUN = serum urea nitrogen; A/G Ratio = albumin/globulin ratio; ALT = alanine amino transferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = γ-glutamyl transferase.

LITERATURE CITED

Bortolotti, A., D. Castelli, and M. Bonati. 1989. Hematology and serum chemistry values of adult, pregnant and newborn New Zealand rabbits (*Oryctolagus cuniculus*). Laboratory Animal Science 39: 437–439.

Jacobson, H. A., and B. S. McGinnes. 1978. Disease and physiologic characteristics of two cottontail populations in Virginia. Wildlife Monograph 60: 5–53.

———, R. L. Kirkpatrick, H. E. Burkhart, and J. W. Davis. 1978. Hematologic comparisons of shot and live trapped cottontail rabbits. Journal of Wildlife Diseases 14: 82–88.

Lepitzki, D. A., and A. Woolf. 1991. Hematology and serum chemistry of cottontail rabbits of southern Illinois. Journal of Wildlife Diseases 27: 643–649.

Lumsden, J. H. 1998. "Normal" or reference values: Questions and comments. Veterinary Clinical Pathology 27: 102–106.

Solberg, H. E. 1983. The theory of reference values. Journal of Clinical Chemistry and Clinical Biochemistry 21: 749–760.

Wells, M. Y., C. P. Decobecq, D. M. Decouvelaere, C. Justice, and P. Guttin. 1999. Changes in clinical pathology parameters during gestation in the New Zealand white rabbit. Toxicologic Pathology 27: 370–379.

Williams, D. F., P. A. Kelly, and L. P. Hamiliton. 1999. Controlled propagation and reintroduction plan for the riparian brush rabbit (*Sylvilagus bachmani riparius*). California State University Stanislaus. http://www.fws.gov/sacramento/es/animal_spp_acct/bunnies/bunny-jump.htm. Accessed January 2009.

Received for publication 25 September 2008.