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Abstract: Complete physical examinations and biomedical sample collection were performed on 70 free-ranging ring-tailed lemurs (*Lemur catta*) from three different habitats in the Beza Mahfaly Special Reserve (BMSR), in southern Madagascar, to assess the impact of humans and habitat on lemur health. Lemurs were chemically immobilized with ketamine and diazepam administered via blow darts for concurrent biomedical, morphometric, and behavioral studies. Subsets of the animals had blood analyzed for hematology, serum chemistry, micronutrients, fat-soluble vitamins (vitamins A, D, and E), measures of iron metabolism, and polymerase chain reaction assays (PCR) for *Toxoplasma gondii*, *Hemoplasma* spp., *Bartonella* spp., *Ehrlichia* spp., *Anaplasma phagocytophilum*, and *Neorickettsia risticii*. Results were compared on the basis of gender and the habitats at the study site: reserve (intact gallery forest), degraded (human inhabited and altered), and marginal (dry didieracea forest with heavy grazing and tree cutting). Levels of vitamin D, triglycerides, and cholesterol, and measures of iron metabolism for BMSR lemurs were greater than those previously reported for a free-ranging lemur population (Tsimanampetsotsa Strict Nature Reserve, Madagascar) with less access to foods of anthropogenic origin. BMSR ring-tailed lemurs from a habitat with less water (marginal) had higher sodium ($P = 0.051$), chloride ($P = 0.045$), osmolality ($P = 0.010$), and amylase ($P = 0.05$) levels than lemurs from other BMSR habitats, suggesting that these lemurs were less hydrated. Vitamin D levels of male lemurs were higher ($P = 0.011$) than those of females at BMSR, possibly because of differences in sunning behavior or differential selection of food items. The biological significance is uncertain for other parameters with statistically significant differences. All samples tested ($n = 20$) were negative for the pathogens tested using PCR assays. Continued concurrent biomedical and ecological research is needed at BMSR to confirm these results and determine their association with population mortality and fecundity rates.

Key words: Ring-tailed lemur, free-ranging, *Lemur catta*, health, habitat, gender.

INTRODUCTION

Biomedical evaluations of free-ranging wildlife have become increasingly common and accepted as tools for improving the health and management of free-ranging populations.[6] Data from biomedical evaluations can be used to assess health and the variation in health among individuals and populations. These data can identify exposure to pathogens or environmental toxicants that are current or potential risks to free-ranging wildlife. In addition, biomedical data can provide information on pathogens and pathogen transmission in free-ranging populations that are, or could become, a threat to human health.[49] Such data can also contribute to improving the health management of captive wildlife populations.[41]

Ring-tailed lemurs (*Lemur catta*) are endemic to southern Madagascar.[37] As with many other species in the suborder Prosimii and other flora and fauna endemic to Madagascar, they are at risk of extinction because of habitat loss.[27] As a consequence, captive propagation programs have been established as one option for conservation of this species. Unlike some prosimian species, ring-tailed lemurs are ecological generalists that have the phys-

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iological and behavioral plasticity to adapt to a range of habitats.[36] Given this plasticity, the ring-tailed lemur's capacity to persist in degraded habitats may serve as a model for how to manage lemur species with more specialized life histories under similar circumstances.

An important part of assessing prospects for ring-tailed lemur persistence in degraded habitats is identifying potential health risks. Biomedical values exist for free-ranging ring-tailed lemurs living within intact, protected reserves.[11] However, the degree to which these biomedical values vary in healthy and diseased free-ranging lemurs in different habitats is less certain. If this can be clarified, these biomedical values may be useful tools for identifying responses to anthropogenic habitat changes.

An earlier report assessed the health of a population of free-ranging ring-tailed lemurs at the Tsimanampetsotsa Strict Nature Reserve, Madagascar (TSNR).[11] This is a relatively isolated protected reserve with little anthropogenic impact, and biomedical parameters and specific pathogens were reported for 20 ring-tailed lemurs without clinical abnormalities. In contrast, this present study reports on values from the Beza Mahafaly Special Reserve, Madagascar (BMSR), a location where there is marked human impact, and includes lemurs with clinical abnormalities. The intent of this study is to develop an understanding of how human and natural factors can influence lemur health. Moreover, the same methods and reference labs were used, if feasible, as the earlier report.[11] This facilitates qualitative comparisons with less concern for differences in methodology.

The BMSR has been the focus of socio-ecological studies of ring-tailed lemurs since 1987.[37] Ring-tailed lemurs inhabiting three different habitats—reserve, degraded, and marginal—were included in this study. The reserve habitat is located within the BMSR, and is an intact gallery forest that has not been disturbed by human activity for over 18 yr. The degraded habitat includes a research camp where a number of Mahafaly families live onsite in dwellings throughout the year, and is a site where researchers intermittently live. This site includes aboveground, uncovered traditional Mahafaly latrines (a Mahafaly taboo prohibits use of inground latrines), trash pits, a covered deep-pit latrine used by non-Mahafaly visitors, a water well, fecal contamination by domestic animals, and grazing by goats and cattle in the surrounding degraded forest.[31,45] Lemurs in this habitat commonly have access to domestic animal and human foods. The marginal habitat is located in a dry didieracea forest approximately 3 km from the gallery forest reserve, and is a site of heavy grazing, domestic animal fecal contamination, and tree cutting.

The objective is to provide a preliminary health assessment of ring-tailed lemurs inhabiting the three different habitats at BMSR and to clarify how human-induced change can alter lemur biomedical parameters. These data may also be of value for interpreting biomedical data in captive ring-tailed lemur populations. This report is a part of a long-term, multidisciplinary study that integrates biomedical assessments with more conventional sociological and ecological studies of BMSR ring-tailed lemurs.

MATERIALS AND METHODS

The study site is the BMSR (23°30′S, 44°40′E), an 80-ha area of land located in southern Madagascar. The climate is temperate with distinct dry (June–September) and wet (October–May) seasons. This study was conducted during the winter dry season because of comparatively easy access to the field site. Ring-tailed lemurs were stratified by habitat. Habitats were classified as reserve (an intact gallery forest), degraded (a site of human habitation), or marginal (a dry didieracea forest with heavy grazing and tree cutting). All protocols were approved by the University of Colorado, Boulder, Institutional Animal Care and Use Committee. Local, Convention on International Trade in Endangered Species of Wild Fauna and Flora (03US04003519), and Centers for Disease Control and Prevention (2003-03-086) permits were obtained for sample collection and transport to the United States.

Sample collection

During June and July 2003, 70 adult and subadult ring-tailed lemurs, 31 males and 39 females, were individually chemically immobilized with darts (Telinject USA, Inc., Agua Dulce, California 91390 USA) prepared the day of immobilization. Darts were delivered through a blow-pipe (Telinject USA, Inc.) by one of two trained, local Mahafaly darters. Immobilizing doses of ketamine hydrochloride (Ketaset, Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA) (20–60 mg total; 12.2–40.5 mg/kg) and diazepam (Valium, Roche Inc., F-92521 Neuilly-s/Seine, CEDEX, France) (0.1–2.0 mg total; 0.05–1.19 mg/kg) were empirically varied to identify doses that provided optimal restraint. Captures followed the May breeding season and all females were presumed to be pregnant.[37] All captures occurred in the morning to permit recovery and release of lemurs before nightfall the same day. Most

lemurs were located in trees when darted; their falls were cushioned by landings in blankets suspended by researchers. Initial respiratory rates were taken, and the lemurs were visually monitored while they were lying on the ground, without contact or stimulation for 5–10 min after darting, until a deep plane of sedation was achieved. Supplemental doses of ketamine or valium were administered as needed for initial restraint, but were generally only administered during subsequent procedures. Saline was applied to the eyes to minimize corneal drying until an ocular exam could be performed, followed by application of an ophthalmic lubricant (Lacrilube, Allergan, Inc., Irvine, California 92623, USA). Lemurs were subsequently moved to a processing site located at the research camp, with sample collection and processing based on previously established protocols (Junge, pers. comm.).[11] Supplemental heat was provided via warm water sealed in elastic gloves for lemurs with body temperatures <38°C. Body weights were measured, and temperature, heart rate, and respiratory rate were monitored every 5–10 min. Neck collars with color-coded numbered tags and subcutaneous microchips (Trovan, Identify UK, Ltd., East Yorkshire, HU13 0RD, United Kingdom) were placed for future identification of lemurs. Complete physical examinations were performed concurrent with morphometric and dental studies, which are reported in detail elsewhere.[4,12,35] Body condition was subjectively classified as poor, good, or obese based on abdominal appearance and prominence of bones with palpation. Representative samples of ectoparasites were collected and placed in 70% ethyl alcohol for subsequent taxonomic identification. Feces were collected for concurrent parasite studies (Hunter-Ishikawa, unpubl. data). Venipuncture of the femoral or jugular vein was performed for blood collection of <1% of the body weight (≤12 ml). Fresh blood was aliquoted to ethylenediaminetetraacetic acid (EDTA) capillary blood collection tubes (Capiject®; Terumo Medical Corporation, Somerset, New Jersey 08873, USA) (0.25 cc), filter paper (3 drops) for polymerase chain reaction (PCR) assays for hemoparasites (Graczyk and Perkins, pers. com.), filter paper (IsoCode® Card, Schleicher and Schuell, Inc., Keene, New Hampshire 03431, USA) for genetic research, heparinized microhematocrit tubes (VWR International, West Chester, Pennsylvania 19380, USA), serum-separator tubes (Becton Dickinson Co., Franklin Lakes, New Jersey 07417 USA), and the fresh preparation of at least four blood smears that were air-dried. Samples placed in serum-separator tubes were kept upright to prevent contamination of blood with zinc from the stopper and protected from direct exposure to sunlight to prevent degradation of fat-soluble vitamins. Lemurs were administered an electrolyte solution (Normosol, Abbott Laboratories, Abbott Park, Illinois USA 60018; 10–30 ml s.c.) for hydration. Skin biopsies were collected from 10 lemurs without clinical abnormalities and placed in formalin to create a reference bank for comparison with lemurs that have dermatological abnormalities and that come from other sites. Lemurs were placed in plastic animal carriers for recovery and released at the site of capture or near their native troop prior to nightfall. All medical waste was burned or otherwise disposed of to minimize contamination of the environment.

Laboratory procedures

Samples were processed within 2 hr of collection. Air-dried blood smears were visually examined to ensure even distribution of a single layer of cells without distortion. At least one slide was left unstained, and at least one was stained with Wright's stain (Harleco, EMD Chemicals, Gibbstown, New Jersey 08027, USA) and preserved with a coverslip and mounting medium. Slides were stored and transported to the United States without exposure to formaldehyde or other fumes that could alter slide quality. A subset of slides ($n = 40$) were examined for hemoparasites and estimates of platelet numbers, and were used for differential white blood cell (WBC) counts by a veterinarian with expertise in clinical hematology (TWC). One hundred white cells were counted at ×100 using an oil-immersion lens for differential WBC counts. Platelets were qualitatively assessed as reduced, adequate, or elevated. At least two microhematocrit tubes were centrifuged at 2,500 rpm for determination of packed cell volume (PCV). Total WBC and red blood cell (RBC) counts (Unopette System, Becton Dickinson Co.) were determined from samples placed in tubes containing EDTA. Unopette kit directions were followed by counting cells in four (WBC) or five (RBC) fields of a hemacytometer (Bright-Line, Fischer Scientific, Houston, Texas 77038 USA). The resulting raw counts were multiplied by 50 (WBC) or 10,000 (RBC) to provide the number of cells/mm^3. Counts for WBC and RBC were conducted twice, and if raw counts varied by more than 20 cells, they were repeated. Raw counts that could not be reconciled were not included in final calculations.

Serum-separator tubes were centrifuged at 4,000 rpm. Serum was divided into at least two cryotubes (Nalge Nunc International, Rochester, New York, 14625, USA), protected from exposure to direct light, and transferred into insulated containers con-

Table 1. Weight and vital signs in free-ranging ring-tailed lemurs from Beza Mahafaly and Tsimanampetsotsa Strict Nature Reserve (TSNR), Madagascar, and captive ring-tailed lemurs (*Lemur catta*).

	Beza Mahafaly			TSNR[11]			Captive[11]	
Parameter	Mean ± SD	Minimum–maximum	*n*	Mean ± SD	Minimum–maximum	*n*	Mean ± SD	*n*
Weight (kg)	2.2 ± 0.2	1.5–2.6[a]	70	1.99 ± 0.34	1.15–2.45	19	—	—
Temperature (°F)	97.1 ± 2.1	90.5–102.5	70	100 ± 0.74	98.2–101.2	18	100.2 ± 1.8	533
Temperature (°C)	36.2 ± 1.2	32.5–39.2	70	37.8 ± 0.4	36.8–38.4	18	37.9 ± 1.0	533
Heart rate (beats/min)	120.9 ± 22.8	76.0–180.0	70	145 ± 27	100–192	20	—	—
Respiration (no./min)	27.0 ± 10.2	12.0–60.0	70	20 ± 6	12–36	18	—	—

[a] Data concurrently published. Adult values only summarized.[35]

taining liquid nitrogen for storage at the research site and transportation to the United States. Samples were stored in a −70°C freezer at the University of Colorado, Boulder. Samples were subdivided for submission to reference laboratories. Reference laboratories for serum chemistry (Antech Diagnostics, Alsip, Illinois 60803, USA), vitamin D (25-hydroxycholecalciferol; Diagnostic Center for Population and Animal Health [DCPAH], Michigan State University, Lansing, Michigan 48909 USA), other fat-soluble vitamins (retinol, retinyl palmitate, retinyl stearate, gamma tocopherol, and alpha tocopherol; Department of Human Nutrition, University of Illinois at Chicago, Chicago, Illinois 60612 USA), trace minerals (DCPAH), iron (Fe), ferritin, and total Fe-binding capacity (TIBC) (Kansas State University, Manhattan, Kansas 66506, USA) were the same as those used for previous research to minimize inter-laboratory variation.[11] Samples for vitamin D and other fat-soluble vitamins were shipped on dry ice, whereas samples sent to other reference laboratories were shipped on ice packs. Twenty lemurs had an additional 0.25 ml whole blood placed in EDTA, frozen in liquid nitrogen for storage and transportation, stored in a −70°C freezer, and assayed in a research laboratory (Veterinary Teaching Hospital, Colorado State University, Fort Collins, Colorado 80523 USA) using PCR assays for *Toxoplasma gondii*, *Hemoplasma* spp., *Bartonella* spp., *Ehrlichia* spp., *Anaplasma phagocytophilum*, and *Neorickettsia risticii*.[18–20,25] Samples were selected based on practical considerations (i.e., sufficient sample volume available and lemurs that appeared to remain within a single habitat type); it was not possible to submit samples from each lemur for all analyses.

Statistical analyses

A commercial software product was used for statistical analyses (Minitab Inc., 3081 Enterprise Drive, State College, Pennsylvania 16801-3008, USA). The distribution of all parameters was summarized (mean, standard deviation, minimum, and maximum). The structure of the data was two-way (habitat by gender); however, only one male each was sampled from the degraded and reserve habitats. Therefore, the analysis was a one-way analysis of variance (ANOVA) comparing habitats and *t*-tests for gender. Deviations from model assumptions for the ANOVA were assessed by examining plots of the residuals.[30] Only lemurs in troops associated with a single habitat type were included in the ANOVA; troops that moved between habitats were excluded. Comparisons were considered statistically significant at $P \leq 0.05$.

RESULTS

Clinical observations included alopecia ($n = 18$); palpable uterine enlargement consistent with pregnancy or other conditions ($n = 4$); poor body condition based on bone prominence ($n = 3$); absent or abnormal digits ($n = 3$); ulcerated and healing subscapular scent glands ($n = 2$); upper respiratory congestion with possible nasolacrimal duct obstruction ($n = 1$); excess seruminous aural exudates ($n = 1$); swollen metatarsus without crepitus or deformity ($n = 1$); poor hair coat ($n = 1$); crepitation and calcification of the distal radius ($n = 1$); pronounced deformation of caudal vertebrae 15 and 16, possibly the result of a healed, misaligned fracture ($n = 1$); severe otitis interna and externa with marked purulent otic discharge, ruptured tympanic membrane, and associated mandibular lymphadenopathy ($n = 1$); a 3/5 S_1 cardiac murmur detected from the ventral, left thoracic cavity, and sinus arrhythmia ($n = 1$); and one individual with genitalia suggestive of hermaphroditism based on the presence of male and female characteristics.

Table 1 summarizes the weights and initial vital

signs for lemurs at the time of capture. There was no difference by habitat for weight ($P = 0.082$), temperature ($P = 0.706$), or heart rate ($P = 0.883$). Weights and initial vital signs did not differ by sex for weight ($P = 0.60$), temperature ($P = 0.12$), heart rate ($P = 0.74$) or respiration ($P = 0.31$).

Table 2 summarizes hematology values conducted in the field (PCV, WBC, RBC) ($n = 59$) and from preserved slides (WBC differential) ($n = 40$) for ring-tailed lemurs. Differentials were not conducted on slides that were required for another study or that did not have sufficient quality to conduct a differential ($n = 19$). There was no difference by habitat for PCV ($P = 0.799$), WBC ($P = 0.876$), neutrophils ($P = 0.108$), lymphocytes ($P = 0.16$), monocytes ($P = 0.222$), or eosinophils ($P = 0.371$). There was no difference by sex for PCV ($P = 0.54$), WBC ($P = 0.537$), neutrophils ($P = 0.83$), lymphocytes ($P = 0.086$), or eosinophils ($P = 0.058$). No statistics were conducted for lemurs with band neutrophils ($n = 4$) and basophils ($n = 3$) because of the small sample size. Two lemurs were qualitatively judged to have reduced platelets, and the remainder ($n = 38$) were judged to have adequate numbers of platelets. Microfilaria were not seen on blood smears.

Table 3 summarizes serum chemistry values. There was no difference by habitat for aspartate aminotransferase (AST) ($P = 0.198$), alanine aminotransferase (ALT) ($P = 0.219$), total bilirubin ($P = 0.438$), alkaline phosphatase (ALP) ($P = 0.121$), gamma glutamyltransferase (GGT) ($P = 0.176$), globulin ($P = 0.515$), albumin:globulin ratio ($P = 0.576$), cholesterol ($P = 0.181$), blood urea nitrogen (BUN) ($P = 0.551$), BUN:creatinine ($P = 0.271$), phosphorus (P) ($P = 0.33$), calcium (Ca) ($P = 0.42$), glucose ($P = 0.781$), lipase ($P = 0.115$), potassium (K) ($P = 0.43$), sodium (Na):K ratio ($P = 0.412$), creatine phosphokinase (CK) ($P = 0.3$), triglycerides ($P = 0.518$), or magnesium (Mg) ($P = 0.173$). There was no difference by sex for AST ($P = 0.13$), ALT ($P = 0.54$), bilirubin ($P = 0.45$), ALP ($P = 0.47$, GGT ($P = 0.17$), globulin ($P = 0.7$), albumin:globulin ratio ($P = 0.59$), cholesterol ($P = 0.39$), BUN ($P = 0.32$), creatinine ($P = 0.071$), BUN:creatinine ratio ($P = 0.34$), P ($P = 0.32$), Ca ($P = 0.14$), glucose ($P = 0.91$), lipase ($P = 0.18$), Na ($P = 0.41$), K ($P = 0.41$), Na:K ratio ($P = 0.4$), CK ($P = 0.079$), triglycerides ($P = 0.089$), or Mg ($P = 0.13$). Values for one animal were not reported for Na, K, P, and Ca.

Table 4 summarizes serum trace minerals, measures of Fe metabolism, and fat-soluble vitamins. There was no difference by habitat for copper (Cu) ($P = 0.251$), zinc (Zn) ($P = 0.125$), vitamin D ($P = 0.266$), retinol ($P = 0.253$), retinyl palmitate ($P = 0.382$), gamma-tocopherol ($P = 0.559$), or alpha-tocopherol ($P = 0.57$). There was no difference based on sex for Cu ($P = 0.1$), Zn ($P = 0.9$), Fe ($P = 0.083$), TIBC ($P = 0.47$), ferritin ($P = 0.19$), retinol ($P = 0.67$), retinyl palmitate ($P = 0.41$), gamma-tocopherol ($P = 0.55$), or alpha-tocopherol ($P = 0.57$). Retinyl stearate was not detected in any samples available for testing ($n = 25$). Values were not reported for one animal for Cu, Zn, chromium (Cr), and boron (B), and sufficient serum was not available for one animal for fat-soluble vitamin assays. Values below detectable ranges for Cr and B precluded statistical analyses for these minerals.

Table 5 summarizes biomedical values with statistically significant differences between male and female lemurs. Table 6 summarizes biomedical values with statistically significant differences between habitats. Each of 20 samples tested for DNA of *Toxoplasma gondii*, *Hemoplasma* spp., *Bartonella* spp., *Ehrlichia* spp., *Anaplasma phagocytophilum*, and *Neorickettsia risticii* by PCR assay was negative.

DISCUSSION

This study documents biomedical data from the first year of a long-term comprehensive study of the health, ecology, behavior, and demography of free-ranging ring-tailed lemurs at BMSR. The focus of this report is to identify variation in biomedical parameters with respect to gender and habitat type. This variation is germane to planning future research on factors that may limit lemur populations in different habitat types. To the extent possible, this study replicated methods that have been previously established at a different field site in Madagascar (TSNR).[11] In particular, the use of the same reference laboratories permits qualitative comparisons of values for serum chemistries, trace minerals, Fe metabolism, and fat-soluble vitamins with TSNR lemurs without consideration for inter-laboratory variation. The remaining parameters reported from BMSR are less directly comparable because of differences in capture and handling, environmental conditions, or equipment calibration. In particular, the use of Jorvet Dip Quick Stain (Jorgensen Laboratories, Loveland, Colorado 80538, USA) for TSNR lemurs and Wright's stain for BMSR lemurs must be considered, because staining of basophilic granules and cytoplasm vary for these two products.[43] This might result in classifying cells differently according to the stain used, and thereby prevent direct comparisons.

The means and 95% confidence intervals of biomedical data are commonly used as indices for bas-

Table 2. Hematology values in free-ranging ring-tailed lemurs from Beza Mahafaly and Tsimanampetsotsa Strict Nature Reserve (TSNR), Madagascar, and captive ring-tailed lemurs (*Lemur catta*).

	Beza Mahfaly			TSNR[11]			Captive[11]	
Parameter	Mean ± SD	Minimum–maximum	*n*	Mean ± SD	Minimum–maximum	*n*	Mean ± SD	*n*
Packed cell volume (%)	43.5 ± 4.5[a]	30.5–52.0	59	38.7 ± 3.6	31–35	20	50.5 ± 6.2	1,249
Red blood cells (10^3/μl or 10^9/L)	6.3 ± 0.9	4.2–8.1	59	—	—			
White blood cells (10^3/μl or 10^9/L)	6.9 ± 2.2	2.8–11.8	59	4.7 ± 1.1	2.7–6.6	20	8.642 ± 3.751	1,226
Neutrophils (%)	38.0 ± 12.5	14.0–75.0	40	49.7 ± 18.3	18–77.5	20	49.4[b]	—
Neutrophils (10^3/μl or 10^9/L)	2.6 ± 1.1	1.2–5.2	40	2.3 ± 1.1	1.04–4.4	20	4.267 ± 2.938	1,083
Band neutrophils (%)	0.1 ± 0.4	0–2	40	0.69 ± 0.69	0–2	20	2.42[a]	—
Band neutrophils (10^3/μl or 10^9/L)	0.013 ± 0.037	0–0.173	40	0.037 ± 0.037	0–0.106	20	0.209 ± 0.226	138
Lymphocytes (%)	48.8 ± 13.1	17.0–68.0	40	41.5 ± 17.9	11–70.5	20	43.4[b]	—
Lymphocytes (10^3/μl or 10^9/L)	3.4 ± 1.3	0.9–6.1	40	2.0 ± 1.1	0.587–3.812	20	3,748 ± 2.141	1,096
Monocytes (%)	8.6 ± 6.3	1.0–32.0	40	4.9 ± 1.6	2–8	20	4.34[b]	—
Monocytes (10^3/μl or 10^9/L)	0.6 ± 0.5	0.1–2.2	40	0.236 ± 0.106	0.086–0.46	20	0.375 ± 0.466	912
Eosinophils (%)	4.4 ± 2.9	0–12.0	40	2.4 ± 2.1	0–6.5	20	3.82[b]	
Eosinophils (10^3/μl or 10^9/L)	0.3 ± 0.3	0–1.1	40	0.1 ± 0.1	0–0.303	20	0.33 ± 0.318	780
Basophils (%)	0.1 ± 0.4	0–2.0	40	0.3 ± 0.5	0–1.5	20	1.77[b]	
Basophils (10^3/μl or 10^9/L)	0.012 ± 0.35	0–0.129	40	0.013 ± 0.018	0–0.055	20	0.153 ± 0.682	70

[a] Data concurrently published.[35]

[b] Calculated from reference values.

ing interpretations of biomedical health.[14] However, data points that are not within reference ranges or otherwise close to the values for the bulk of the population are of interest for their significance in clinical and free-ranging settings. In clinical settings, such ''abnormal'' results are of value for interpreting pathologic conditions, guiding additional diagnostics and therapeutics, and providing prognoses. In addition to their diagnostic value, extreme biomedical values for free-ranging animals are of interest where they can be associated with variations in ecological conditions, particularly if corresponding changes in mortality and fecundity rates can be documented. The focus of this study was to evaluate associations between biomedical values and ecological conditions, rather than to establish reference ranges.

Gender and troop membership are potential confounders in the statistical analyses for habitat effects, because data from only one male each were available from the degraded and reserve habitats, and all or most individuals from each habitat were members of the same troop. Therefore, it is not possible to determine whether differences between habitats could be attributable to differences in gender, and a one-way ANOVA was conducted rather than a two-way ANOVA. These confounders and the opportunistic nature of sampling indicate that the biological interpretations of these results are best viewed as preliminary and as the basis for designing future studies.

Intramuscular administration of diazepam has long been recognized as a valuable adjunct to ketamine hydrochloride immobilization of wild animals because of its anticonvulsant and muscle relaxant properties, even though diazepam is slowly absorbed with intramuscular administration.[13,29] Other immobilizing agents and combinations for immobilization of wild animals have been developed since the first use of ketamine–diazepam immobilization protocols. However, because of the limited availability of drugs at this remote field location during this field season, ketamine and diazepam were judged the best immobilizing agents available. This was based on minimal cardiopulmonary effects on lemurs and drug stability under field conditions. Diazepam is known to adsorb to plastics.[29] However, this is primarily applicable to intravenous administration of this drug. Adsorption to plastic syringes and darts is trivial for the length of time diazepam contacted plastic syringes and darts during our study.[48] As with all immobilization procedures, there are always various risks to consider and minimize to the extent possible. Immobilization methods have the potential to alter hematology and plasma biochemistry values; however, there is a paucity of literature on the effects of immobilization methods on lemur hematology or plasma biochemistry values.[17,32]

Many of the results obtained were similar to those from TSNR.[11] However, differences in hematology and serum chemistry results between TSNR and BMSR were observed, as were differences based on gender and habitat type at BMSR (Tables 1–6). Such differences were not unexpected, given the high number of parameters considered, and the relatively small subpopulations analyzed for this report.[33] Therefore, although qualitative comparisons with TSNR lemurs or statistically significant differences among BMSR lemurs may exist for some parameters, the biological significance is uncertain for differences among single parameters that are not supported with additional data. Consequently, it is most appropriate to defer discussion of these parameters until their biological significance is confirmed. Of greater interest and the focus of this discussion are parameters that are often considered complementary for clinical interpretation. In such instances, the use of multiple parameters that share a similar clinical interpretation provides greater confidence that biological differences among groups of BMSR lemurs are truly being documented.

Although BMSR lemurs appeared to be heavier (Table 1) and have higher WBC counts than values previously reported for lemurs at TSNR (Table 2), it is difficult to make direct comparisons of data in Tables 1 and 2 for the two sites, because of differences in methods and equipment. Cholesterol and triglyceride values were measured by the same laboratory and can be compared. Cholesterol and triglyceride values for BMSR lemurs are intermediate between those of TSNR and captive populations (Table 3). Although pathologic levels of cholesterol and triglycerides have not been defined for lemurs, elevations in cholesterol or triglycerides have been associated with metabolic abnormalities and systemic disease in other species.[42] It is possible that the intermediate values of cholesterol and triglycerides for BMSR lemurs represent their greater access to nonnative foods, as compared with TSNR lemurs. However, this would need to be confirmed with further research.

Hemosiderosis has been observed in captive lemurs, and was believed to be because of diets high in ascorbic acid and low in tannins.[39] Because lemurs at BMSR have access to food items consumed by humans and domestic animals, indices of Fe metabolism were compared for lemurs from different habitats to investigate the potential for access to

Table 3. Serum chemistry values in free-ranging ring-tailed lemurs from Beza Mahafaly and Tsimanampetsotsa Strict Nature Reserve (TSNR), Madagascar, and captive ring-tailed lemurs (*Lemur catta*).

	Beza Mahfaly			TSNR[11]			Captive[11]	
Parameter	Mean ± SD	Minimum–maximum	(*n*)	Mean ± SD	Minimum–maximum	(*n*)	Mean ± SD	(*n*)
AST[a] (IU/L)	64.6 ± 45.1	24–208	27	70.7 ± 62.3	13–216	20	48 ± 37	816
ALT[b] (IU/L)	53.6 ± 11.2	39–89	27	44.8 ± 14.8	22–73	20	94 ± 59	763
Total bilirubin (mg/dL)	0.4 ± 0.1	0.3–0.7	27	0.2 ± 0.08	0.1–0.4	20	0.6 ± 0.4	838
Total bilirubin (μmol/L)	6.7 ± 1.6	5.1–12.0	27	4.2 ± 1.4	1.7–6.8	20	10 ± 7	838
ALP[c] (IU/L)	114.1 ± 42.2	58–205	27	60.5 ± 17.1	35–94	20	222 ± 109	864
GGT[d] (IU/L)	13.4 ± 3.7	7–25	27	16.3 ± 4.1	10–25	20	28 ± 18	483
Total protein (g/dL)	5.8 ± 0.7	4.7–7.8	27	5.6 ± 0.71	4.3–7.3	20	7.3 ± 0.8	755
Total protein (g/L)	58 ± 7	47–78	27	55.6 ± 7.1	43–73	20	73 ± 8	755
Albumin (g/dL)	4.3 ± 0.4[e]	3.3–5.4[e]	27	4.27 ± 0.43	3.5–5.3	20	5.7 ± 0.9	673
Albumin (g/L)	43 ± 4	33 ± 54	27	42.7 ± 4.3	35–53	20	57 ± 9	673
Globulin (g/dL)	1.5 ± 0.4	0.9–2.6	27	1.29 ± 0.42	0.6–2	20	1.6 ± 0.9	660
Globulin (g/L)	15 ± 4	9 ± 26	27	12.9 ± 4.2	6–20	20	16 ± 9	660
Albumin:globulin[f]	3.1 ± 0.7	1.9–4.8	27	3.5 ± 1.1	2.4–5.8	19	—	—
Cholesterol (mg/dL)	60.4 ± 15.3	41–114	27	44.3 ± 7.4	30–56	19	89 ± 26	768
Cholesterol (mmol/L)	1.56 ± 0.4	1.06–2.96	27	1.15 ± 0.19	0.8–1.5	20	2.3 ± 0.7	768
BUN[g] (mg/dL)	7.3 ± 6.0	5–34	27	13.3 ± 4.5	5–20	20	22 ± 8	903
BUN (mmol/L)	2.6 ± 2.14	1.78–12.1	27	4.75 ± 1.6	1.8–7.1	20	7.9 ± 2.9	903
Creatinine (mg/dL)	0.8 ± 0.2	0.5–1.2	27	0.9 ± 0.2	0.5–1.3	19	1 ± 0.3	891
Creatinine (μmol/L)	72 ± 14	44.2–106.8	27	78.1 ± 19.6	44.2–115	19	88 ± 27	891
BUN:creatinine[h]	9.3 ± 7.0	4–38	27	15.3 ± 6.5	6–29	20	—	—
P[i] (mg/dL)	5.0 ± 0.9	3.6–6.6	26	4.6 ± 1.1	2.3–6.5	20	5.4 ± 2	734
P (mmol/L)	1.6 ± 0.3	1.2–2.1	26	1.5 ± 0.4	0.7–2.1	20	1.7 ± 0.7	734
Ca[j] (mg/dL)	8.1 ± 0.5	7–9.6	26	8.0 ± 1.1	5.9–10.1	20	9.7 ± 0.9	851
Ca (mmol/L)	1.9 ± 0.1	1.7–2.3	26	2.0 ± 0.3	1.5–2.5	20	2.4 ± 0.2	851
Glucose (mg/dL)	188.6 ± 80.1	85–396	27	136 ± 36.9	67–211	20	142 ± 0.2	901
Glucose (mmol/L)	10.5 ± 4.4	4.7–22.0	27	7.6 ± 2.1	3.7–11.7	20	7.88 ± 4.2	901
Amylase (IU/L)	1,034.5 ± 182.6	702–1,454	27	808 ± 167	511–1,058	20	1,779 ± 770	263
Lipase (IU/L)	45.1 ± 31.1	25–180	27	54.6 ± 23.7	25–101	18	66 ± 106	134
Na[k] (mEq/L)	145.0 ± 6.7	137–172	26	137 ± 12.7	117–156	20	148 ± 5	747
K[l] (mEq/L)	3.3 ± 0.6	2–4.4	26	4.0 ± 1.0	2.6–5.8	20	4.4 ± 0.6	754
Na:K	44.4 ± 8.8	32–74	26	35.8 ± 6.1	23–45	20	—	—
Cl[m] (mEq/L)	103.4 ± 4.0	93–109	27	99.7 ± 12	79–119	20	108 ± 6	740
CK[n] (IU/L)	4,508.8 ± 2,959.9	1,671–14,450	27	3,288 ± 1,790	1,298–6,694	17	1,363 ± 1,248	259
Triglycerides (mg/dL)	43.0 ± 10.4	26–75	27	20.8 ± 7.0	10–35	20	69 ± 35	472

Table 3. Continued.

Parameter	Beza Mahfaly			TSNR[11]			Captive[11]	
	Mean ± SD	Minimum–maximum	(*n*)	Mean ± SD	Minimum–maximum	(*n*)	Mean ± SD	(*n*)
Triglycerides (mmol/L)	0.5 ± 0.12	0.3–0.8	27	0.2 ± 0.08	0.1–0.4	20	0.8 ± 0.4	472
Osmolality[o] (milliosmoles/kg)	300.7 ± 8.0	283–322	27	287 ± 26.3	241–322	20	298 ± 10	45
Magnesium (mg/dL)	2.2 ± 0.3	1.7–2.6	27	2.2 ± 0.3	1.58–2.8	20	2.02 ± 0.47	67

[a] AST = aspartate aminotransferase.
[b] ALT = alanine aminotransferase.
[c] ALP = alkaline phosphatase.
[d] GGT = gamma glutamyltransferase.
[e] Data concurrently published.[35]
[f] Albumin to globulin ratio calculated using conventional units (g/dl).
[g] BUN = blood urea nitrogen.
[h] BUN:creatinine ratio calculated using conventional units (mg/dl).
[i] P = phosphorus.
[j] Ca = calcium.
[k] Na = sodium.
[l] K = potassium.
[m] Cl = chloride.
[n] CK = creatinine phosphokinase.
[o] Osmolality (calculated).

Table 4. Serum trace minerals, measures of iron metabolism, and fat-soluble vitamins in free-ranging ring-tailed lemurs from Beza Mahafaly and Tsimanampetsotsa Strict Nature Reserve (TSNR), Madagascar, and captive ring-tailed lemurs (*Lemur catta*).

	Beza Mahfaly			TSNR[11]			Captive[11]	
Parameter	Mean ± SD	Minimum–maximum	*n*	Mean ± SD	Minimum–maximum	*n*	Mean ± SD	*n*
Cu[a] (μg/dL)	102.7 ± 17.1	75–137	26	76.5 ± 21	35.9–109	18	—	—
Cu (μmol/L)	16.2 ± 2.7	11.8–21.6	26	12.0 ± 3.3	5.6–17.1	18	—	—
Zn[b] (μg/dL)	90.0 ± 21.7	5.9–121	26	65.7 ± 24.3	31.7–112	18	—	—
Zn (μmol/L)	13.8 ± 3.3	0.9–18.5	26	10.1 ± 3.7	4.85–17.1	18	—	—
Cr[c]	All values <10 ug/dl		26	—	—		—	—
B[d]	All values <100 ug/dl		26	—	—		—	—
Fe[e] (μg/dL)	155.9 ± 41.8[f]	75.0–278.0	27	71.4 ± 31.9	28–132	18	261 ± 95	60
Fe (μmol/L)	27.9 ± 7.5	13.4 ± 49.8	27	12.8 ± 5.7	5–23.6	18	46.7 ± 17	60
TIBC[g] (μg/dL)	308.5 ± 43.9	241.0–395.0	27	241 ± 37.4	192–326	18	—	—
TIBC (μmol/L)	55.2 ± 7.9	43.2–70.7	27	43.1 ± 6.7	34.4–58.4	18	—	—
Ferritin (ng/dL)	169.4 ± 157.3	40.0–754.0	27	40.9 ± 22.5	16–99	17	—	—
Ferritin (μmol/L)	385 ± 357.6	90.9–1713.6	27	91.9 ± 50.6	36–222	17	—	—
Vitamin D (ng/ml)	34.1 ± 14.8	14.0–67.0	27	11.3 ± 4.9	4.8–23.2	20	—	—
Vitamin D (μmol/L)	85.1 ± 36.9	34.9–167.2	27	28.1 ± 12.3	12–58	20	—	—
Retinol (μg/dL)	21.6 ± 3.7	15.7–29.7	26	18.9 ± 5.2	10.5–31.6	20	—	—
Retinol (μmol/L)	0.75 ± 0.13	0.55–1.04	26	0.66 ± 0.18	0.37–1.1	20	—	—
Retinyl palmitate (μg/dL)	1.9 ± 1.0	0.8–4.3	26	—	—	20	—	—
Retinyl palmitate (μmol/L)	0.04 ± 0.02	0.02–0.08	26	—	—	20	—	—
Gamma-tocopherol (μg/dL)	18.1 ± 5.4	10.0–30.0	26	15.6 ± 5.8	9–27	16	—	—
Gamma-tocopherol (μmol/L)	0.43 ± 0.13	0.24–0.72	26	0.37 ± 0.14	0.22–0.65	16	—	—
Alpha-tocopherol (μg/dL)	184.8 ± 55.9	101.0–299.0	26	195 ± 59.8	99–331	20	—	—
Alpha-tocopherol (μmol/L)	4.3 ± 1.3	2.3–6.9	26	4.5 ± 1.4	2.3–7.68	20	—	—

[a] Cu = copper.
[b] Zn = zinc.
[c] Cr = chromium.
[d] B = boron.
[e] Fe = iron.
[f] Data concurrently published.[35]
[g] TIBC = total iron binding capacity.

Table 5. Biomedical values with statistically significant differences between male and female ring-tailed lemurs (*Lemur catta*) at Beza Mahafaly, Madagascar.

Parameter	Female		Male		Female versus male ($P < 0.050$)
	Mean ± SD	*n*	Mean ± SD	*n*	
Monocytes (%)	9.82 ± 6.72	28	5.67 ± 3.68	12	$P = 0.017$
Eosinophils(10^3/μl or 10^9/L)	0.4 ± 0.3	15	0.2 ± 0.1	8	$P = 0.048$
Red blood cells (10^3/μl or 10^9/L)	5.97 ± 0.94	32	6.61 ± 0.72	27	$P = 0.004$
Total protein (g/dL)	5.96 ± 0.76	19	5.46 ± 0.33	8	$P = 0.026$
Total protein (g/L)	59.6 ± 7.6	19	54.6 ± 3.3	8	$P = 0.026$
Albumin (g/dL)	4.48 ± 0.46	19	4.04 ± 0.19	8	$P = 0.002$
Albumin (g/L)	44.8 ± 4.6	19	40.4 ± 1.9	8	$P = 0.002$
Amylase (IU/L)	982 ± 156	19	1,158 ± 191	8	$P = 0.042$
Chloride (mEq/L)	102.3 ± 4.4	18	105.8 ± 1.5	8	$P = 0.007$
Osmolality (milliosmoles/kg)	298.6 ± 8.1	18	305.4 ± 5.7	8	$P = 0.024$
Vitamin D (ng/ml)	28.0 ± 8.04	19	48.7 ± 17.2	8	$P = 0.011$
Vitamin D (μmol/L)	69.8 ± 20.1	19	121.6 ± 42.9	8	$P = 0.011$

anthropogenic sources of food to influence lemur health. Significant differences in serum Fe ($P = 0.004$), TIBC ($P = 0.01$), and ferritin ($P = 0.006$) were observed for lemurs from different habitats, with the highest levels of Fe and TIBC in lemurs from the degraded habitat, and highest levels of ferritin in the marginal habitat (Table 6). The means for each measure of Fe metabolism from each habitat were also greater than those previously reported at TSNR (Table 4).[11] However, these levels were

Table 6. Biomedical values in ring-tailed lemurs (*Lemur catta*) with statistically significant differences in degraded (D), marginal (M), and reserve (R) habitats at Beza Mahafaly, Madagascar.

Parameter	D		M		R		C versus M versus R ($P < 0.05$)
	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	
Respiration (no./min)	36.2 ± 10.3	9	25.0 ± 7.2	11	28.7 ± 8.9	6	$P = 0.029$
Red blood cells (10^3/μl or 10^9/L)	5.52 ± 0.88	7	6.78 ± 0.76	11	6.31 ± 0.94	6	$P = 0.019$
Total protein (g/dL)	6.3 ± 0.79	10	5.47 ± 0.53	11	5.7 ± 0.41	6	$P = 0.025$
Total protein (g/L)	63.0 ± 7.9	10	54.7 ± 5.3	11	57.0 ± 4.1	6	$P = 0.025$
Albumin (g/dL)[a]	4.7 ± 0.35	10	4.1 ± 0.35	11	4.3 ± 0.39	6	$P = 0.002$
Albumin (g/L)	47.0 ± 3.5	10	41.0 ± 3.5	11	43.0 ± 3.9	6	$P = 0.002$
Creatinine (mg/dL)	0.8 ± 0.14	10	0.8 ± 0.13	11	1.0 ± 0.13	6	$P = 0.007$
Creatinine (μmol/L)	70.7 ± 12.4	10	70.7 ± 11.5	11	88.4 ± 11.5	6	$P = 0.007$
Amylase (IU/L)	944 ± 140	10	1,132 ± 175	11	1,006 ± 197	6	$P = 0.050$
Na[b] (mEq/L)	142.8 ± 3.5	9	148.6 ± 8.7	11	141.7 ± 2.3	6	$P = 0.051$
Cl[c] (mEq/L)	102.3 ± 4.6	9	105.6 ± 2.4	11	101.0 ± 4.1	6	$P = 0.045$
Osmolality[d] (milliosmoles/kg)	297.4 ± 7.1	9	305.9 ± 7.4	11	295.8 ± 4.9	6	$P = 0.010$
Fe (μg/dL)[a,e]	188.3 ± 41.3	10	134.2 ± 31.3	11	141.7 ± 25.7	6	$P = 0.004$
Fe (μmol/L)	33.7 ± 7.4	10	24.0 ± 5.6	11	25.4 ± 4.6	6	$P = 0.004$
TIBC[f] (μg/dL)	338.3 ± 21.9	10	283.3 ± 38.1	11	305.2 ± 55.5	6	$P = 0.010$
TIBC (μmol/L)	60.6 ± 3.9	10	50.7 ± 6.8	11	54.6 ± 9.9	6	$P = 0.010$
Ferritin (ng/dL)	100.0 ± 57.4	10	278.3 ± 193.5	11	85.5 ± 61.4	6	$P = 0.006$
Ferritin (μmol/L)	227.3 ± 130.5	10	632.5 ± 439.8	11	194.3 ± 139.5	6	$P = 0.006$

[a] Data concurrently published.[35]
[b] Na = sodium.
[c] Cl = chloride.
[d] Osmolality (calculated).
[e] Fe = iron.
[f] TIBC = total iron-binding capacity.

less than values considered pathologic in other species.

The interpretations of the measures of Fe metabolism are based on work from other species.[26] Serum Fe is a measure of Fe^{3+} bound to serum transferin, which is a transport molecule, and can be an indication of Fe absorption from the diet.[46] Serum transferin is measured as TIBC, which represents the maximum amount of Fe that can bind to transferin. Ferritin appears to be the best serum measure of body stores of Fe, but requires use of a species-specific immunologic assay that has not been validated for ring-tailed lemurs.[2,26] These assays are used as indices of body stores of Fe with a particular interest in identifying pathologic levels. However, levels of Fe in liver are considered the gold standard for body levels of Fe, and have not been standardized with serum levels of Fe, TIBC, or ferritin in lemurs.[46] Further uncertainty in interpretation of these results is created by factors that may influence serum Fe metabolite levels, such as inflammation, fasting, stress, and levels of other minerals.[26] Therefore, it cannot be established whether the indices of Fe metabolism reported are pathologic. If they do represent abnormal levels, differences between study sites could be because of differences in consumption of natural or anthropogenic foods, direct or indirect consumption of soils with different levels of Fe or other minerals, or other factors.[10,35] Further work is needed to validate the diagnostic value of these indices of Fe metabolism, identify whether consumption of excess Fe occurs, and document whether there is an association between serum levels of Fe, TIBC, or ferritin, individually or in combination, with changes in lemur activity, mortality, or fecundity.

Serum vitamin D (25-hydroxycholecalciferol) levels of male lemurs at BMSR were greater than those of females at BMSR ($P = 0.0011$) (Table 5). The mean vitamin D level of BMSR lemurs was approximately three times greater than those of TSNR (Table 4) without corresponding abnormalities in Ca or P levels (Table 3). This difference was evident for both sexes (Table 5), but males averaged more than four times the group mean at TSNR. The magnitude of these differences warrants further consideration.

Hypotheses to explain differences between BMSR and TSNR populations include differing levels of exposure to ultraviolet (UV) light, dietary factors, and differences in sample handling.[44] Differing levels of exposure to UV light and dietary factors are among the possible explanations that could account for differences in vitamin D levels for male and female lemurs at BMSR. The period of time when samples were collected from BMSR and TSNR included the austral winter solstice and the lowest UV exposure of the year. This suggests that the levels of solar radiation at the time of sample collection do not account for higher levels of vitamin D in BMSR ring-tailed lemurs. BMSR is colder than TSNR.[22] Therefore, because lemurs compensate for low metabolic rates by employing sunning behavior to raise body temperatures, differences in sunning behavior need to be investigated as a possible explanation for comparatively high levels of vitamin D in BMSR lemurs.

Differential consumption of food items with high levels of one or more forms of vitamin D is an alternate explanation that could account for the present observations. Elevations in vitamin D metabolites have been observed in captive common marmosets (*Callithrix jacchus*) and brown lemurs (*Eulemur fulvus*) fed diets high in vitamin D, and selection of food items high in vitamin D could account for differences between BMSR and TSNR lemurs.[16,38] It is not clear whether storage of samples at different temperatures (−20°C in a non–frost-free freezer for TSNR versus −70°C for this report) could account for the differences between populations.[11] However, based on canine vitamin D concentrations with different sample handling conditions, it is unlikely that differences in storage temperatures can fully account for differences between lemur populations (Nachreiner, pers. comm.). Further work is needed to clarify the basis for what appear to be substantial differences in vitamin D levels between genders at BMSR and between populations.

Ring-tailed lemurs in marginal habitats had relatively higher values for Na ($P = 0.051$), chloride ($P = 0.045$), and osmolality ($P = 0.01$) (Table 6). Changes in serum osmolality often parallel changes in serum Na, and thus osmolality is not an entirely independent measure of hydration status.[42] However, these values may reflect physiological responses of lemurs to environments with limited water availability. This matches observations of available water resources in the different habitats; less water is available in the marginal habitat.[34] It may be relevant that lemurs from the marginal habitat also have higher levels of amylase than lemurs from the other habitats (Table 6); decreased glomerular filtration could result in hyperamylasemia, as well as the elevations in Na, chloride, and osmolality values.[42] Alternative explanations for hyperamylasemia include pancreatitis, intestinal disease, and hepatic disease. Although dietary and other factors could account for these latter explanations, the relatively low predictive value for am-

ylase values for other species in clinical settings limits confidence in these explanations without concurrent supporting evidence.

Based on overlapping ranges or differences between means that are clinically trivial for individuals (i.e., total protein 59.6 g/L for females and 54.6 g/L for males), it is less certain that other statistically significant differences in values between genders (Table 5) are biologically significant. However, the higher values for total protein and albumin for females at BMSR may be an indication that the nutritional plane of females is greater, as would be expected of a female-dominated social hierarchy. Similar to the differences between genders, higher values for total protein and albumin levels in lemurs from degraded habitat may be a biologically significant consequence of consuming food of human origin (Table 6). The difference ($P = 0.019$) in RBC between habitats is of uncertain significance. This uncertainty is compounded by the error associated with manual counts, although manual counts were the only option for this field location.[8]

Small sample sizes prevent characterization of specific clinical abnormalities with corresponding biomedical data. However, individuals with clinical abnormalities were observed with atypical biomedical values that characterize these abnormalities. Lemur 148 had otitis interna and externa, a ruptured tympanic membrane, mandibular lymphadenopathy, and profuse purulent otic discharge. This lemur was anemic and had a relative neutrophilia, as would be expected given the clinical abnormalities; lemur 148 had the lowest values for PCV (30.5%) and lymphocytes (17%), as well as the highest percentage of neutrophils (75%) and total WBC ($11.8 \times 10^3/\mu l$) in the population sampled. It is worth noting that 1 yr later (June 2004), with the infection still unresolved and draining into the oral cavity, limited jaw mobility, and the presence of a solid mass near the distal end of the left mandibular ramus (in the area of the temporomandibular joint), lemur 148 had gained 180 g and appeared to be an active member of the population (Sauther and Cuozzo, pers. comm.). Clinical abnormalities associated with lemur 188 (from marginal habitat) included a high degree (56%) of tooth loss, numerous skin lesions, white multifocal discoloration of the tongue, a subjectively high ectoparasite load noted on gross physical examination, and a relatively light body weight (1.62 kg) for an adult ring-tailed lemur at BMSR.[4] This individual was anemic (PCV = 32), hypoalbuminemic (33 g/L), and azotemic (BUN = 34 mg/dL, BUN:creatinine = 38, osmolality = 322 mmol/kg), with some of the most extreme values for these parameters among the population sampled. Finally, individual 156 (from degraded habitat) was a marked individual known to be 16 yr old, and the oldest lemur sampled.[34] This individual's values for AST (117 IU/L), ALP (205 IU/L), total protein (76 g/L), and globulin (26 g/L) were among the highest, and its albumin:globulin ratio (1.9) the lowest of the lemurs sampled. Although the ALT values (50 IU/L) were not atypical, the relative hyperglobulinemia, hyperproteinemia, and elevated AST and ALP suggest the possibility of an inflammatory process with possible hepatic involvement. It is also possible that elevated AST and ALP levels could be because of concurrent muscle or bone pathology, respectively. Given the age and habitat of this individual, such findings would not be surprising if confirmed by necropsy or other methods. Data associated with these three individuals support the use of these biomedical methods to identify individuals with clinical abnormalities, and are consistent with the use of these methods to identify biologically significant differences between populations of lemurs. Although individuals such as lemur 148 can survive with marked clinical abnormalities, the relative importance of these clinical observations and atypical biomedical values in these individuals is best viewed with respect to population level effects on mortality and fecundity rates. These data may also be relevant for evaluating the health of captive lemurs.

Vitamin A (retinol, retinyl palmitate) and vitamin E (alpha- and gamma-tocopherol) levels were similar to those of animals from TSNR (Table 4), suggesting that the dietary intake of these compounds was similar for both populations. Retinyl palmitate was extremely low in both populations. Retinyl stearate was below the level of detection, likely because of the rapid disappearance of retinyl esters from the bloodstream and storage in the liver of this species, as is the case with other primate species. Vitamin A and vitamin E levels appeared to be low compared to other primate species.[3] Further work is needed to clarify whether these levels are ''normal,'' establish the levels of these compounds in the diet, determine lemur's capability to absorb and utilize these compounds, and identify population-level impacts associated with variable levels of these fat-soluble vitamins.

Mg, Zn, Cu, B, and Cr are trace minerals, and are believed to be essential for normal mammalian metabolism. The levels of Mg, Zn, and Cu found in this study were not markedly different from those of TSNR lemurs and were not significantly different among habitats and gender at BMSR; B and Cr levels have not previously been reported for

lemurs. These minerals may serve as markers of heavy metal exposure for free-ranging ring-tailed lemurs. Although these minerals were not detected at levels that would suggest heavy metal exposure, observations that lemurs commonly lick the walls of painted structures as well as other behaviors (Sauther, pers. comm.) suggest that the potential for exposure exists. Further study is warranted for these and other heavy metals such as lead.

PCR was utilized to screen for *Toxoplasma gondii*, *Hemoplasma* spp., *Bartonella* spp., *Ehrlichia* spp., *Anaplasma phagocytophilum*, and *Neorickettsia risticii* in 20 lemurs as an exploratory analysis.[19] The failure to amplify DNA from these organisms suggests that active infection was not occurring at the time these lemurs were sampled. The results of these assays can be falsely negative in infected animals because of variation in DNA quantities in individual blood samples. It is also possible that these pathogens are present in the population, but not detected, because of the relatively small number of animals sampled. Serology would be a complementary method to employ with PCR, because this could identify animals that have been infected with the agent but have resolved or low-level infections. Little is known of these agents in Madagascar.[7,40] However, they are widely distributed in Africa and Asia.[1,15,23,24] Because Madagascar has had a number of agents introduced with the immigration of humans and animals, if these agents are not presently in Madagascar, there is potential for introduction in the future with the import of live animals or migration of people.[9,28] Because definitive hosts (i.e., cats for *T. gondii*), potential vectors (i.e., mesostigmatid mites found on lemurs), and human and domestic animal reservoirs are present in lemur habitat, lemurs could be exposed to these agents if present in Madagascar. There is evidence that some of these agents can infect lemurs.[21,47] Although there is much interest in emerging pathogens as a general topic, it is not clear whether these agents could become emerging pathogens that pose a threat to free-ranging lemur populations.[5]

CONCLUSIONS

The results of this study must be considered preliminary. However, apparent differences in biomedical parameters between groups of lemurs may reflect on-going biological processes. Similarly, atypical biomedical values that characterize specific clinical abnormalities were observed for some individuals. Differences among groups in measures of Fe metabolism and serum lipids may represent exposure to anthropogenic factors that are health risks to lemur populations. Differences in Na, Cl, and osmolality among lemurs inhabiting different habitats may reflect physiological responses to natural environmental variation. Elevated levels of vitamin D in BMSR lemurs compared with TSNR lemurs, and elevated levels in male lemurs at BMSR relative to females are of uncertain biological significance. Further work is needed to determine the degree to which various anthropogenic and natural factors affect lemur mortality or fecundity rates, and the degree to which biomedical parameters can be used to characterize these processes. If these biomedical parameters prove to be valid indicators of physiological or pathological processes in lemur populations, they can be used to identify risks to lemur populations, evaluate alternate management strategies, and serve as survey tools for assessing population health.

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LITERATURE CITED

1. Allsopp, M. T. E. P., M. Louw, and E. C. Meyer. 2005. *Ehrlichia ruminantium*—an emerging human pathogen. SAMJ (S. Afr. Med. J.) 95: 541.

2. Andrews, G. A., P. S. Chavey, L. M. Mazzaro, J. L. Dunn, and D. J. St. Aubin. 2004. Enzyme-linked immunosorbent assay to quantitate serum ferritin in the northern fur seal (*Callorhinus ursinus*). Zoo Biol. 23: 79–84.

3. Crissey, S. D., J. E. Barr, K. A. Slifka, P. E. Bowen, M. Stacewicz-Sapuntzakis, C. Langman, A. Ward, and K. Ange. 1999. Serum concentrations of lipids, vitamins A

and E, vitamin D metabolites, and carotenoids in nine primate species at four zoos. Zoo Biol. 18: 551–564.

4. Cuozzo, F. P., and M. L. Sauther. 2004. Tooth loss, survival, and resource use in wild ring-tailed lemurs (*Lemur catta*): implications for inferring conspecific care in fossil hominids. J. Hum. Evol. 46: 623–631.

5. Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious disease of wildlife: threats to biodiversity and human health. Science 287: 443–449.

6. Deem, S. L., W. B. Karesh, and W. Weisman. 2001. Putting theory into practice: wildlife health in conservation. Conserv. Biol. 15: 1224–1233.

7. Dromigny, J. A., J. L. Pecarrere, F. Leroy, G. Ollivier, and P. Boisier. 1996. Approche de la prevalence de la toxoplasmose à Tanarive. Bull. Soc. Pathol. Exotic 89: 212–216.

8. Duncan, J. R., and K. W. Prasse. 1977. Veterinary Laboratory Medicine, 2nd ed. Iowa State Univ. Press. Ames, Iowa. P. 8.

9. Duplantier, J.-M., and J.-B. Ducheman. 2003. Human diseases and introduced small mammals. *In*: Goodman, S. M., and J. P. Benstead (eds.). The Natural History of Madagascar. Univ. Chicago Press, Chicago, Illinois. Pp. 158–161.

10. Du Puy, D. J., and J. Moat. 2003. Using geologic substrate to identify and map primary vegetation types in Madagascar and the implications for planning biodiversity conservation. *In*: Goodman, S. M., and J. P. Benstead (eds.). The Natural History of Madagascar. Univ. Chicago Press, Chicago, Illinois. Pp. 51–67.

11. Dutton, C. J., R. E. Junge, and E. E. Louis. 2003. Biomedical evaluation of free-ranging ring-tailed lemurs (*Lemur catta*) in Tsimanampetsotsa Strict Nature Reserve, Madagascar. J. Zoo Wildl. Med. 34: 16–24.

12. Fish, K. D., M. L. Sauther, and F. P. Cuozzo. 2004. Effects of habitat on fluctuating asymmetry in a population of wild ring-tailed lemurs (*Lemur catta*). Am. J. Phys. Anthropol. 38: 94–95.

13. Fowler, M. E. 1978. Restraint. *In*: Fowler, M. E. (ed.). Zoo and Wild Animal Medicine. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 35–52.

14. Galen, R. S., and R. S. Gambino. 1975. Beyond Normality: The Predictive Value and Efficiency of Medical Diagnoses. John Wiley and Sons, New York, New York.

15. Giroud, P., J. Jadin, B. Fiocre, M. Capponi, N. Dumas, and A. Ryter. 1970. En pays divers, Afrique centrale, orientale, Madagascar, Iran, Sardaigne, France, chez des animaux parasités par des *Anaplasma*, des *Babesia*, des *Theileria*, on constate des sérologies positives sur le groupe Néorickettsien (*Bedsonia*, *Cytoecetes Phagocytophilla*). Bull. Soc. Pathol. Exotic Filiales 63: 630–635.

16. Gray, T. K., G. E. Lester, G. Moore, D. Crews, E. L. Simons, and M. Stuart. 1982. Serum concentrations of calcium and vitamin D metabolites in prosimians. J. Med. Primatol. 11: 85–90.

17. Heard, D. J., and V. J. Huft. 1998. The effects of short-term physical restraint and isoflurane anesthesis on hematology and plasma biochemistry in the island flying fox (*Pteropus hypomenlanus*). J. Zoo Wildl. Med. 29: 14–17.

18. Homan, W. L., M. Vercammen, J. De Braekeleer, and H. Verschueren. 2000. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. Int. J. Parasitol. 30: 69–75.

19. Jensen, W. A., M. R. Lappin, S. Kamker, and W. Reagan. 2001. Use of a polymerase chain reaction assay to detect and differentiate two strains of *Haemobartonella felis* in naturally infected cats. Am. J. Vet. Res. 62: 604–608.

20. Jensen, W. A., M. Z. Rooney, J. Rooney, D. Kordickm, and E. Breitschwerdt. 2000. Rapid identification and differentiation of *Bartonella* species using a single-step PCR assay. J. Clin. Microbiol. 38: 1717–1722.

21. Junge, R. 2003. Prosimians. *In:* Fowler, M. E., and R. E. Miller (eds.). Zoo and Wild Animal Medicine, 5th ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 334–346.

22. Jury, M. R. 2003. The climate of Madagascar. *In:* Goodman, S. M., and J. P. Benstead (eds.). The Natural History of Madagascar. Univ. Chicago Press, Chicago, Illinois. Pp. 75–87.

23. Kelly, P. J., G. N. Eoghain, and D. Raoult. 2004. Antibodies reactive with *Bartonella henselae* and *Ehrlichia canis* in dogs from communal lands of Zimbabwe. J. S. Afr. Vet. Assoc. 75: 116–120.

24. Kusiluka, L. J. M., B. Ojeniyi, N. F. Friis, B. Kokotovic, and P. Ahrens. 2001. Molecular analysis of field strains of *Mycoplasma capricolum* subspecies *capripneumoniae* and *Mycoplasma mycoides* subspecies *mycoides*, small colony type isolated from goats in Tanzania. Vet. Microbiol. 82: 27–37.

25. Lappin, M. R., E. B. Breitschwerdt, W. A. Jensen, B. Dunnigan, J. Rha, C. R. Williams, M. Brewer, and M. Fall. 2004. *Anaplasma phagocytophilum* infection of cats in North America. J. Am. Vet. Med. Assoc. 225: 893–896.

26. Lowenstine, L. J, and L. Munson. 1999. Iron overload in the animal kingdom. *In:* Fowler, M. E. and R. E. Miller (eds.). Zoo and Wild Animal Medicine, Current Therapy, 4. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 260–269.

27. Mittermeier, R. A., W. R. Konstant, M. E. Nicoll, and O. Langrand. 1992. Lemurs of Madagascar: an action plan for their conservation. IUCN/SSC Primate Specialist Group. IUCN Publications Services Unit, 219c Huntingdon Road, Cambridge CB3 0DL, United Kingdom. 58 pp.

28. Nozais, J. P. 2003. The origin and dispersion of human parasitic diseases in the old world (Africa, Europe and Madagascar). Mem. Inst. Oswald. Cruz. 98(Suppl. 1): 13–19.

29. Plumb, D. C. 2005. Veterinary Drug Handbook, 5th ed. Blackwell Publishing, Ames, Iowa. P. 236.

30. Ramsey, F. L., and D. W. Schafer. 1997. The Statistical Sleuth: A Course in Methods of Data Analysis. Duxbury Press, Belmont, California.

31. Ratsirarson, J. 2003. Réserve Spéciale de Beza Mahafaly. *In:* Goodman, S. M., and J. P. Benstead (eds.). The

Natural History of Madagascar. Univ. Chicago Press, Chicago, Illinois. Pp. 1520–1525.

32. Rietkerk, F. E., E. C. Delima, and S. M. Mubarak. 1994. The hematological profile of the mountain gazelle (*Gazella gazelle*): variations with sex, age, capture method, season, and anesthesia. J. Wildl. Dis. 30: 69–76.

33. Sackett, D. L, B. R. Haynes, G. H. Guyatt, and P. Tugwell. 1991. Clinical Epidemiology, a Basic Science for Clinical Medicine. Little, Brown, and Co., Boston, Massachusetts.

34. Sauther, M. L., and F. P. Cuozzo, Somatic variation in living, wild ring-tailed lemurs (*Lemur catta*). Folia Primatol. (In Press).

35. Sauther, M. L., K. D. Fish, F. P. Cuozzo, D. S. Miller, M. Hunter-Ishikawa, and H. Culbertson. 2006. Patterns of health, disease, and behavior among wild ring-tailed lemurs, *Lemur catta*: effects of habitat and sex. *In:* Jolly, A., R.W. Sussman, N. Koyama, and H. Rasamimanana (eds.). Ring-tailed Lemur Biology. Springer, New York, New York. Pp. 313–331.

36. Sauther, M. L., and R. W. Sussman. 1993. A new interpretation of the organization and mating systems of the ring-tailed lemur (*Lemur catta*). *In:* Kappeler, P. M., and J. Ganzhorn (eds.). Lemur Social Systems and Their Ecological Basis. Plenum Press, New York, New York. Pp. 11–121.

37. Sauther, M. L., R. W. Sussman, and L. Gould. 1999. The socioecology of the ringtailed lemur: thirty-five years of study. Evol. Anthropol. 8: 120–132.

38. Shinki, T. Y. Shiina, N. Takahashi, Y. Tanioka, H. Koizumi, and T. Suda. 1983 Extremely high circulating levels of 1α,25-dihydroxyvitamin D_3 in the marmoset, a New World monkey. Biochem. Biophys. Res. Commun. 114: 452–457.

39. Spelman, L. H., K. G. Osborn, and M. P. Anderson. 1989. Pathogenesis of hemosiderosis in lemurs: role of dietary iron, tannin, and ascorbic acid. Zoo Biol. 8: 239–251.

40. Sureau, P., J. P. Raynaud, C. Lapeire, and E. R. Brygoo. 1962. Premier isolement de *Toxoplasma gondii* a Madagascar. Toxoplasmose spontanée et expérimentale du *Lemur catta*. Bull. Soc. Pathol. Exotic Filiales. 55: 357–362.

41. Terio, K. A., L. Marker, and L. Munson. 2004. Evidence for chronic stress in captive but not free-ranging cheetahs (*Acinonyx jubatus*) based on adrenal morphology and function. J. Wildl. Dis. 40: 259–266.

42. Thrall, M. A., D. C. Baker, T. W. Campbell, D. DeNicola, M. J. Fettman, E. D. Lassen, A. Rebar, and G. Weiser. 2004. Veterinary Hematology and Clinical Chemistry. Lippincott Williams and Wilkins. Baltimore, Maryland.

43. Tvedten, H., and R. L. Cowell. 1999. Cytology of neoplastic and inflammatory masses. *In:* Willard, M. D., H. Tvedten, and G. H. Turnwald (eds.). Small Animal Clinical Diagnosis by Laboratory Methods, 3rd ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 310–323.

44. Ullrey, D. E. 2003. Metabolic bone diseases. *In:* Fowler, M. E., and R. E. Miller (eds.). Zoo and Wild Animal Medicine, 5th ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 749–756.

45. Whitelaw, D., M. L. Sauther, J. E. Loudon, and F. Cuozzo. 2005. Anthropogenic change in and around Beza-Mahafaly Reserve: methodology and results. Am. J. Phys. Anthropol. 40(Suppl.): 222.

46. Williams, C. V., J. Campbell, and K. M. Glenn. 2006. Comparison of serum iron, total iron binding capacity, ferritin, and percent transferrin saturation in nine species of apparently healthy captive lemurs. Am. J. Primatol. 68: 477–489.

47. Williams C. V., J. L. Van Steenhouse, J. M. Bradley, S. I. Hancock, B. C. Hegarty, and E. B. Breitschwerdt. 2002. Naturally occurring *Ehrlichia chaffeensis* infection in two prosimian primate species: ring-tailed lemurs (*Lemur catta*) and ruffed lemurs (*Varecia variegata*). Emerg. Infect. Dis. 8: 1497–1500.

48. Winsnes, M., R. Jeppsson, and B. Sjoberg. 1981. Diazepam adsorption to infusion sets and plastic syringes. Acta Anaesthesiol. Scan. 25: 93–6.

49. Wolfe, N. D., A. A. Escalante, W. B. Karesh, A. A. Kilbourn, and A. Lal. 1998. Wild primate populations in emerging infectious disease research: the missing link? Emerg. Infect. Dis. 4: 149–158.

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