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COMPARISON OF BIOMEDICAL EVALUATION FOR WHITE-FRONTED BROWN LEMURS (*EULEMUR FULVUS ALBIFRONS*) FROM FOUR SITES IN MADAGASCAR

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Abstract: Health and nutritional assessments of wildlife are important management tools and can provide a means to evaluate ecosystem health. Such examinations were performed on 37 white-fronted brown lemurs (*Eulemur fulvus albifrons*) from four sites in Madagascar. Comparison of health parameters between sites revealed statistically significant differences in body weight, body temperature, respiratory rate, hematology parameters (white cell count, hematocrit, segmented neutrophil count, and lymphocyte count), serum chemistry parameters (aspartate aminotransferase, alanine aminotransferase, serum alkaline phosphatase, total protein, albumin, phosphorus, calcium, sodium, chloride, and creatinine phosphokinase), and nutrition parameters (copper, zinc, ferritin, retinol, tocopherol, and 25-hydroxycholecalciferol). Two of 10 lemurs tested were positive for toxoplasmosis; none of 10 were positive for *Cryptosporidium* or *Giardia*. Enteric bacteria and endo- and ectoparasites were typical. Statistically different values in hematology and chemistry values probably do not reflect clinically significant differences, whereas nutrition parameter differences are likely related to season, soil, and forage availability.

Key words: Lemur, health, nutrition, Madagascar, conservation medicine, disease ecology.

INTRODUCTION

Madagascar is considered one of the world's conservation priorities because of rapid habitat destruction and expanding human population. As habitats become fragmented islands, animal populations are isolated, and stochastic events such as disease outbreaks may have catastrophic effects.[6,8,33,47] Evaluation and monitoring of animal health and nutrition (conservation medicine) is critical for the management and preservation of endangered species.[7,28,34,42]

The white-fronted brown lemur (*Eulemur fulvus albifrons*) is a medium-sized (2–2.6 kg), cathemeral lemur species restricted to northeastern Madagascar; it is exclusively arboreal, with a frugivorous–folivorous diet.[11] The taxonomy of the *E. fulvus* group is under discussion;[11,36,46] therefore, the historical designation of the subspecies will be used. The subspecies is threatened by both habitat destruction and hunting for bush meat.[36] Habitat destruction may result in isolation of populations by preventing emigration/immigration. Such isolation may reduce genetic diversity and affect health and survival. With a species that exists in multiple populations, health and nutritional status may also reflect local conditions. Significant differences between populations would suggest differences in local conditions, possibly alerting managers to risks to the population.

Evaluation of free-ranging populations of wild animals is an important management tool for assessing the health of the species and ecosystem, as well as to identify disease risks, nutritional status, and changes in these parameters. Such health assessments have been completed on some primate species,[29,38,39] including assessments of human impact on wild populations.[24,37,40] Health and nutrition assessments have been done on several lemur species.[10,12,19–23,35] Parameters from healthy wild populations should be used as reference values; however, care must be taken to confirm that the wild population is in fact healthy and representative. The four areas from which lemurs were sampled in this project represent variations of habitat, human interaction, degree of isolation, and north–south extent of *E. f. albifrons* range. Variations within health and nutrition parameters may reflect these variations.

MATERIALS AND METHODS

Lemurs evaluated in this report were from four protected areas: Betampona Special Reserve (BSR,

From the St. Louis Zoo, 1 Government Drive, St. Louis, Missouri 63110, USA (Junge); Toronto Zoo, 361A Old Finch Avenue, Toronto, Ontario M1B 5K7, Canada (Dutton); Denver Zoological Gardens, 2300 Steele Street, Denver, Colorado 80205, USA (Knightly); Duke Lemur Center, 3705 Erwin Road, Durham, North Carolina 27705, USA (Williams); University of Antananarivo, Antananarivo, Madagascar (Rasambainarivo); and Henry Doorly Zoo, 3701 South 10th Street, Omaha, Nebraska 68107, USA (Louis). Present address (Rasambainarivo): Parc Ivoloina, Tamatave, Madagascar. Correspondence should be directed to Dr. Junge (Junge@stlzoo.org).

17°54′S, 49°13′E, 10 animals), Marojejy National Park and World Heritage Site (MJNP, 14°25′S, 49°45′E, 7 animals), Masoala National Park and World Heritage Site (MNP, 15°35′S, 50°10′E, 10 animals), and Nosy Mangabe Special Reserve (NMSR, 15°30′S, 49°46′S, 10 animals). Betampona is 2,228 hectares of low-altitude rainforest surrounded by agricultural areas, and is an isolated population of *E. f. albifrons* at the southernmost extent of the range. Marojejy National Park is 60,150 hectares with a wide variety of microclimates, has the highest rainfall on the island, and includes both low-altitude and midaltitude rainforest. Masoala National Park is one of the largest protected areas in Madagascar, and covers 210,200 ha of coastal and lowland rainforest, on the peninsula that forms the northern coast of the Bay of Antongil. Nosy Mangabe Special Reserve is a 520-hectare island covered with low-altitude rainforest, much of which is secondary growth and represents an isolated population.

Sample collection

Data were collected as part of an ongoing prosimian biomedical research project. This project is structured to provide collaboration between field biologists and veterinarians in the field. Veterinarians provide basic medical assistance as needed, and collect standard medical samples and information from animals anesthetized or captured for other purposes. Thirty-seven white-fronted brown lemurs (17 males, 20 females) were individually anesthetized with the use of tiletamine and zolazepam (Telazol®, Fort Dodge Animal Health, Overland Park, Kansas 66225, USA; 10 mg/kg, i.m.) by dart (Type C Disposable Dart, Pneu-Dart, Williamsport, Pennsylvania 17701, USA). Rectal temperature, heart rate, respiratory rate, and body weight were measured; a complete physical examination was performed; and blood, fecal, and ectoparasite samples were collected. Each animal was monitored by assessing heart rate, respiratory rate, and body temperature. Each animal was given subcutaneous balanced electrolyte solution (Lactated Ringer's Solution, LRS) equivalent to the amount of blood collected. Animals were held in cloth bags until they fully recovered from anesthesia, and then they were released at the site of capture.

Blood samples were collected; the samples did not exceed 1% of body weight (1 ml/100 g body weight). Whole blood (0.5 ml) was immediately transferred into ethylenediaminetetraacetic acid (EDTA) anticoagulant, and the remaining volume was transferred into nonanticoagulant tubes and allowed to clot. Serum tubes were centrifuged within 4 hr of collection. Serum was pipetted into plastic tubes and frozen in liquid nitrogen for transport. Once transported to the St. Louis Zoo (St. Louis, Missouri, USA), the samples were stored at -70°C until analysis.

Fecal samples were collected either from freshly voided feces, or from the rectum. Samples could not be obtained from all animals. Approximately 1 cc of feces were placed into a transport medium (SAF Fixative, Remel Company, Lenexa, Kansas 66215, USA) for examination for parasite ova. If sufficient feces were obtained, a second 1-cc sample was frozen in liquid nitrogen for bacterial culture. External parasites discovered on physical examination were removed with a cotton swab or forceps and placed into 95% ethyl alcohol. For all numeric parameters, mean ± SD values are reported. Parameters were compared for significant differences between sexes and between sites. No values from captive animals were available for comparison.[18] Initially, all means were compared with the use of Bonferroni all-pairwise multiple-comparison analysis of variance (ANOVA); then those parameters that were statistically different ($P < 0.05$) were confirmed via a two-sample *t*-test (Number Cruncher Statistical Systems, Kaysville, Utah 84037, USA).

Laboratory procedures

Within 4 hr of collection (usually 2 hr), two blood-smear slides were made from each anticoagulant sample. These smears were fixed, stained, and preserved with coverslips and mounting medium for later evaluation. A total white blood cell count was done within 8 hr of collection (Unipette System, Becton Dickenson Company, Franklin Lake, New Jersey 07417, USA). Stained smears were examined microscopically for differential blood cell count and hemoparasite examination.

Serum was submitted to the indicated laboratories for the following analyses: serum biochemical profile (AVL Veterinary Laboratories, St. Louis, Missouri 63139, USA), toxoplasmosis titer (University of Tennessee Comparative Parasitology Service, Knoxville, Tennessee 37996, USA), 25-hydroxycholecalciferol (vitamin D) and trace mineral analysis (Animal Disease Diagnostic Laboratory, Lansing, Michigan 48910, USA), fat-soluble vitamin analysis (University of Illinois Nutrition Laboratory, Chicago, Illinois 60612, USA), and iron metabolism analysis (Kansas State University, Manhattan, Kansas 66506, USA). Fecal samples in transport medium were submitted for examination for parasites and ova by standard centrifugation techniques. Fecal cultures were submitted by thaw-

Table 1. Physical examination parameters for *Eulemur fulvus albifrons* from four sites in Madagascar.

	Betampona Special Reserve (n = 10)	Marojejy National Park (n = 7)	Masoala National Park (n = 7)	Nosy Mangabe Special Reserve (n = 10)
Weight (kg)	2.05 ± 0.3[a]	2.31 ± 0.3[a]	1.57 ± 0.2[b]	1.68 ± 0.2[b]
Temp (°C)	38.6 ± 0.8[a]	37.4 ± 0.9[b]	38.3 ± 0.9[a,b]	37.4 ± 1.0[b]
Pulse (per min)	235 ± 37[a]	223 ± 37[a]	220 ± 24[a]	218 ± 26[a]
Respirations (per min)	73 ± 27[a]	75 ± 36[a]	39 ± 21[b]	45 ± 12[a,b]

[a,b] Values within a row with different superscripts are statistically different ($P < 0.05$).

ing the frozen fecal samples and inoculating a culture transport swab (Copan Diagnostics, Corona, California 91716, USA). These swabs were submitted for aerobic culture; specifically *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* identification was requested. Samples were plated on XLD agar, *Campylobacter* agar, SS agar, MacConkey agar, *Yersinia* agar, Brilliant Green agar, and blood agar, and in selenite broth to enrich for *Salmonella* and *Shigella*. After incubation in selenite broth, samples were replated on XLD agar, HE agar, SS agar, Brilliant Green agar, and MacConkey agar. If sufficient feces were available, samples were submitted for *Giardia* antigen detection (University of Miami, Miami, Florida 33136, USA) and *Cryptosporidium* polymerase chain reaction (PCR) (Centers for Disease Control, Atlanta, Georgia, USA). Ectoparasites were submitted for identification.

RESULTS

Thirty-seven individuals were examined—10 animals (5 males, 5 females) from BSR (April 2006), 7 animals (4 males, 3 females) from MJNP (June 2003), 10 animals (5 males, 5 females) from MNP (October 2002), and 10 animals (3 males, 7 female) from NMSR (October 2004). One animal from MNP was a juvenile; the remaining 36 individuals were adults. Three animals from BSR had significant injuries. One adult male was missing 1 cm of the upper lip margin lateral to the left canine; this was presumed to be a fight wound. One adult female was missing the distal 5 cm of her maxillae bilaterally, including upper canines and incisors and external nares, resulting in an open nasal cavity. One adult male had a 5-cm wound in the right abdominal wall, which had a loop of intestine adherent and exposed externally. Physical examination results are summarized in Table 1, with the juvenile animal excluded from mean weight calculation. No significant differences exist between males and females for any parameter or site. Significant differences were noted between sites for weight, body temperature, and respiratory rate.

Complete blood cell counts and biochemical profiles

Results are presented in Tables 2 and 3. No significant differences exist between males and females for any blood cell count or serum chemistry parameter or site. For complete blood cell counts, significant differences exist between sites for total white cell count, hematocrit, percent segmented neutrophil leukocytes, and percent lymphocytes. For biochemical profiles, significant differences between sites exist for aspartate aminotransferase, alanine aminotransferase, serum alkaline phosphatase, total protein, albumin, phosphorus, calcium, sodium, chloride, and creatine phosphokinase. No hemoparasites were identified.

Serology

Ten samples (four from BSR, six from NMSR) were submitted for *Toxoplasma* serology (IgG). Two animals from NMSR had positive titers to toxoplasmosis (1:32, 1:128).

Nutritional assessment

Trace-mineral evaluations were performed on 27 individuals, and fat-soluble vitamin evaluations were performed on 28 individuals. Results are presented in Tables 4 and 5. No significant difference exists between males and females for any parameter. Significant differences for copper, zinc, ferritin, retinol, tocopherol, and 25-hydroxycholecalciferol were detected between sites.

Fecal examinations

Enteric cultures were done on 20 individuals. The following organisms were identified: Group D *Enterococcus*, *Enterobacter*, *Bacillus* spp., *Escherichia coli*, coagulase-negative *Staphylococcus*, *Citrobacter freundii*, *Acinetobacter*, *Pseudomonas*, *Staphylococcus intermiditis*, *Klebsiella oxytoca*, and *Lactobacillus*. Fecal samples from 10 individuals were submitted for *Giardia* antigen detection and *Cryptosporidium* detection by PCR (eight from

Table 2. Complete white blood cell count and differential count values for *Eulemur fulvus albifrons* from four sites in Madagascar.

	Betampona Special Reserve (n = 10)	Marojejy National Park (n = 7)	Masoala National Park (n = 9)	Nosy Mangabe Special Reserve (n = 10)
White blood cells (1,000/μl)	10.0 ± 4.7[a]	10.1 ± 1.1[a]	10.1 ± 4.1[a]	4.6 ± 1.7[b]
Hematocrit (%)	47.6 ± 9.4[a,b]	51.3 ± 2.6[a]	40.1 ± 14.0[a,b]	37.8 ± 2.9[b]
Segmented neutrophils (%)	51.4 ± 15.7[a,b]	39.3 ± 13.4[a]	63.8 ± 14.2[b]	46.6 ± 8.1[a]
Segmented neutrophils (per μl)	5,140 ± 1,570[a,b]	3,969 ± 1,350[a]	6,444 ± 1,430[b]	214 ± 37[a]
Band neutrophils (%)	0[a]	0.1 ± 0.4[a]	0.4 ± 0.7[a]	0[a]
Band neutrophils (per μl)	0[a]	1 ± 4[a]	4 ± 7[a]	0[a]
Lymphocytes (%)	43.6 ± 14.6[a,b]	59.3 ± 13.8[b]	29.1 ± 10.9[a]	50.3 ± 10.2[b]
Lymphocytes (per μl)	4,360 ± 1,460[a,b]	5,989 ± 1,394[b]	2,939 ± 1,101[a]	2,314 ± 469[b]
Eosinophils (%)	0[a]	0.4 ± 0.8[a]	2.9 ± 3.2[a]	0.2 ± 0.6[a]
Eosinophils (per μl)	0[a]	40 ± 8[a]	293 ± 323[a]	9 ± 28[a]
Monocytes (%)	1.4 ± 1.5[a]	0.4 ± 0.5[a]	3.1 ± 2.2[a]	1.8 ± 2.9[a]
Monocytes (per μl)	140 ± 150[a]	40 ± 51[a]	310 ± 222[a]	83 ± 133[a]
Basophils (%)	0[a]	0[a]	0[a]	0[a]
Basophils (per μl)	0[a]	0[a]	0[a]	0[a]

[a,b] Values within a row with different superscripts are statistically different ($P < 0.05$).

NMSR, two from BSR), and all were negative for both pathogens.

Mites were present on all lemurs examined at BSR, MJNP, and MNP; however, none were found at NMSR. These mites are classified in the family Laelapidae, but the genus has not been described. Mites were frequently present in the external ears and groin area, but also elsewhere on the body. Mite infestation was not associated with evidence of pruritus, alopecia, or abnormal hair. Fecal parasite exams were performed on samples from 29 individuals (10 from BSR, 2 from MJNP, 7 from MNP, and 10 from NMSR) and enteric parasites were present in 20 individuals; these are reported in Table 6.

DISCUSSION

The data presented here allow an assessment of differences that exist between isolated populations of *E. f. albifrons* that include habitat types representing much of the range of the species in eastern Madagascar. Samples were collected in April and October, introducing seasonal and climactic variables. In eastern Madagascar, day length (approximately 12 hr), is nearly equal for these months and similar temperature ranges exist (April low–high = 21–29°C; October low–high = 19–27°C).[49] However, rainfall is quite different in these months. April is shortly after the rainy season, yet an average of 320 mm of rainfall occurs. October is in the middle of the dry season, receiving 120 mm (average) rainfall.[49] Although no significant differences were detected between sexes, differences in body weight exist between populations, with BSR (mean = 2.05 kg) and MJNP (mean = 2.31 kg) differing from MNP (mean = 1.57 kg) and NMSR (mean = 1.68 kg). This could reflect nutritional status, health status, or genetic differences between populations. Differences noted in body temperature and respiratory rate are most likely related to plane of anesthesia. Statistically significant differences exist within hematology parameters, but no clear explanation or pattern is discernible. Although mean values are statistically different, it is likely that they represent normal variation that would lose significance in a larger sample size rather than clinically important differences in health.

For serum chemistries, significant differences were noted in the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum alkaline phosphatase (SAP); protein values (total protein and albumin); electrolytes calcium, sodium, and chloride; and in the muscle enzyme creatinine phosphokinase (CPK). As was found with hematology values, no consistent pattern was apparent for the serum chemistry parameters. Values for liver enzymes AST and ALT were similar for BSR and MNP, and for MJNP and NMSR, with significant differences in values between the pairs. It is likely that these value differences reflect sample size or possibly handling methods rather than clinically significant differences. Although care was taken to process and freeze samples quickly, and to maintain them properly frozen, it is possible that improper handling occurred, which may alter values.[15,17]

Table 3. Serum biochemical profile values for *Eulemur fulvus albifrons* from four sites in Madagascar.

	Units	Betampona Special Reserve (n = 10)	Marojejy National Park (n = 7)	Masoala National Park (n = 6)	Nosy Mangabe Special Reserve (n = 10)
Aspartate aminotransferase	IU/L	26.3 ± 41.5[a]	113.9 ± 59[b]	94.5 ± 50.7[b]	24.6 ± 28.2[a]
Alanine aminotransferase	IU/L	40.3 ± 19.3[a]	89.3 ± 24.1[b]	86.5 ± 33.9[b]	40.8 ± 30.7[a]
Total bilirubin	mg/dl	0.4 ± 0.2[a]	0.2 ± 0.1[a]	0.2 ± 0.1[a]	0.4 ± 0.3[a]
	μmol/L	6.8 ± 3.4[a]	3.4 ± 1.7[a]	3.4 ± 1.7[a]	6.8 ± 5.1[a]
Serum alkaline phosphatase	IU/L	191.6 ± 131.1[a]	71.1 ± 19.4[b]	217.4 ± 37.7[a]	59.9 ± 29.1[b]
Gamma glutamyltransferase	IU/L	17.1 ± 3.3[a]	11.3 ± 9.3[a]	15.5 ± 9.8[a]	18.8 ± 5.1[a]
Total protein	g/dl	7.2 ± 0.8[a,b]	8.0 ± 1.0[b]	6.8 ± 0.4[a] (n = 8)	6.7 ± 0.6[a]
	g/L	72 ± 8[a,b]	80 ± 10[b]	68 ± 4[a]	67 ± 6[a]
Albumin	g/dl	4.9 ± 0.8[a]	6.2 ± 1.0[b]	5.0 ± 0.5[a,b]	4.3 ± 0.6[a]
	g/L	49 ± 8[a,b]	62 ± 10[b]	50 ± 5[a,b]	43 ± 6[a]
Globulin	g/dl	2.3 ± 0.9[a]	1.9 ± 0.4[a]	1.8 ± 0.2[a]	2.4 ± 0.7[a]
	g/L	23 ± 9[a]	19 ± 4[a]	18 ± 2[a]	24 ± 7[a]
Blood urea nitrogen	mg/dl	8.8 ± 9.0[a]	8.0 ± 3.3[a]	7.3 ± 4.3[a]	8.7 ± 7.3[a]
	mmol/L	3.1 ± 3.2[a]	2.9 ± 1.2[a]	2.6 ± 1.5[a]	3.1 ± 2.6[a]
Creatinine	mg/dl	0.9 ± 0.1[a]	1.2 ± 0.2[a]	1.4 ± 1.0[a]	1.1 ± 0.4[a]
	μmol/L	79.6 ± 8.8[a]	106.1 ± 17.7[a]	123.8 ± 88.4[a]	97.2 ± 35.4[a]
Phosphorus	mg/dl	5.2 ± 1.1[b]	3.3 ± 1.4[a]	4.3 ± 1.1[a,b]	4.4 ± 1.4[a,b]
	mmol/L	1.7 ± 0.4[b]	1.1 ± 0.5[a]	1.4 ± 0.4[a,b]	1.4 ± 0.5[a,b]
Calcium	mg/dl	9.5 ± 1.0[a,b]	10.6 ± 0.8[a]	9.7 ± 0.9[a,b]	9.3 ± 0.7[b]
	mmol/L	2.4 ± 0.3[a,b]	2.7 ± 0.2[a]	2.4 ± 0.2[a,b]	2.3 ± 0.2[b]
Glucose	mg/dl	75.7 ± 62[a]	66.9 ± 53.9[a]	27.8 ± 43.7[a]	46.2 ± 58.6[a]
	mmol/L	4.2 ± 3.4[a]	3.7 ± 3.0[a]	1.5 ± 2.4[a]	2.5 ± 3.2[a]
Amylase	IU/L	Nd[c]	2,467 ± 741[a]	2,155 ± 482[a]	Nd
Lipase	IU/L	Nd	119 ± 98[a]	132 ± 62[a]	Nd
Sodium	mEq/L = mmol/L	137.0 ± 2.7[a,b]	148.8 ± 9.2[a,b]	153.3 ± 14.7[c]	137.0 ± 4.4[b]
Potassium	mEq/L = mmol/L	4.7 ± 1.0[a]	5.0 ± 0.5[a]	5.4 ± 0.5[a]	5.2 ± 0.5[a]
Chloride	mEq/L = mmol/L	99.9 ± 3.1[a]	107.7 ± 7.1[a]	117.7 ± 11.3[b]	106.8 ± 3.8[a,b]
Creatinine phosphokinase	IU/L	790.5 ± 305[a]	8,901 ± 7,913[b] (n = 6)	5,690 ± 2,865[a,b]	991 ± 254[a]
Triglycerides	mg/dl	Nd	31.7 ± 14.9[a]	31.0 ± 12.4[a]	Nd
Magnesium	mg/dl	Nd	2.5 ± 0.2[a]	2.5 ± 0.2[a]	Nd
	mmol/L	Nd			Nd

[a,b] Values within a row with different superscripts are statistically different ($P < 0.05$).
[c] Nd = Analysis not done.

Table 4. Serum trace minerals and iron analytes for *Eulemur fulvus albifrons* from four sites in Madagascar.

	Betampona Special Reserve (n = 10)	Marojejy National Park (n = 5)	Masoala National Park (n = 2)	Nosy Mangabe Special Reserve (n = 10)
Copper (μg/dl)	147 ± 38[a]	Nd[c]	Nd	94 ± 17[b]
Zinc (μg/dl)	104 ± 47[a]	220 ± 90[b]	84 ± 1[a]	80 ± 23[a]
Iron (μg/dl)	156.9 ± 123.3[a]	111.6 ± 32.7[a]	85.0 ± 49.9[a]	58.1 ± 59.7[a]
Total iron-binding capacity (μg/dl)	305.9 ± 64.6[a]	340.6 ± 135.0[a]	283.5 ± 40.3[a]	323.7 ± 77.7[a]
Ferritin (ng/ml)	153.5 ± 96.1[a]	127 ± 124[a]	521.5 ± 403.8[b]	295.2 ± 112.9[a,b]
Transferrin saturation (%)	48.3 ± 30.3[a]	36.1 ± 14.4[a]	31.6 ± 22.0[a]	48.4 ± 17.0[a]

[a,b] Values within a row with different superscripts are statistically different ($P < 0.05$).
[c] Nd = analysis not performed.

Table 5. Fat-soluble vitamins[a] for *Eulemur fulvus albifrons* from four sites in Madagascar.

	Betampona Special Reserve (n = 10)	Marojejy National Park (n = 6)	Masoala National Park (n = 2)	Nosy Mangabe Special Reserve (n = 10)
25-hydroxycholecalciferol (μg/dl)	141.8 ± 35.8[b]	52.0 ± 20.4[c]	117 ± 50.1[b,c]	103.4 ± 25.0[b,c]
Retinol (μg/dl)	13.8 ± 4.7[b]	11.7 ± 5.0[b]	9.6 ± 1.7[b,c]	4.4 ± 2.7[c]
Retinyl palmitate (μg/dl)	0.2 ± 0.6[b]	0.2 ± 0.6[b]	0.3 ± 0.7[b]	0[b]
Gamma tocopherol (μg/dl)	26.8 ± 6.6[b]	43.3 ± 16.5[c]	12.7 ± 8.4[d]	14.7 ± 4.5[d]
Alpha tocopherol (μg/dl)	153.4 ± 55.5[b]	289.3 ± 10.4[c]	90.5 ± 43.8[b,d]	51.2 ± 27.8[d]

[a] Retinyl sterate and carotenoids (alpha carotene, beta carotene, cryptoxanthin, lycopene) not detected in any sample.
[b,c,d] Values within a row with different superscripts are statistically different ($P < 0.05$).

Trace mineral, iron analyte, and fat-soluble vitamin differences indicate variation in dietary intake. Detailed analysis of nutrient composition of dietary items is not available to confirm these differences. Variation in nutrient composition may be due to soil composition, plant selection, or season. In addition, small sample size (n = 2 for MNP) makes critical comparison difficult. Copper and zinc values vary between sites, yet are within the range considered typical for mammals[27] and as seen with other lemur species.[10,21–23]

Serum iron values are significantly higher from sites visited in April than in October, probably reflecting changes in vegetation consumed. Total iron-binding capacity and transferrin saturation are statistically similar between sites, whereas ferritin values vary. Serum iron analyte determination is a useful measure of iron metabolism, deficiency, or excess.[50] Values for *E. f. albifrons* fall within general mammal ranges (serum iron 55–185 μg/dl, total iron-binding capacity [TIBC] 250–425 μg/dl, transferrin saturation [T sat] 33%).[31] Serum iron analytes are used to reflect body iron stores in humans and may be applied to some animal species[31,44]; however, recent data suggest that the value of these parameters in lemurs varies markedly between species.[50,51] Evidence of iron deposition in the liver (hemosiderosis) of captive lemurs suggests that intake exceeds requirement on commonly fed captive diets. The extrapolation has been made to suggest that this contributes to significant hepatic disease (hemochromatosis);[45] however, recent research suggests that iron deposition and iron analyte analysis must be evaluated with caution.[13,50,51] Ferritin is an intracellular storage molecule for iron and in some species correlates well to total body iron stores. Transferrin is the plasma transport molecule for iron, and the percent saturation is considered a reflection of iron absorption (but not an accurate measure of iron stores), which is related to dietary intake. TIBC measures the maximum amount of iron that can be transported. It has been suggested that these parameters can be applied to lemurs in the same manner as humans to assess iron status; however, more thorough investigation[50,51] has revealed that there is marked species variability and normal reference ranges must be determined for each species. Using a traditional interpretation, this would suggest that absorption and dietary iron level are similar between populations (similar transferrin saturation), whereas body stores (correlated to ferritin levels) vary by site. Unless dietary iron levels had been high in previous years (resulting in excess storage) and lower in the current year (resulting in typical transferrin saturation), this explanation seems unlikely. The variation in iron analytes is a very important observation in light of the interest in iron metabolism in captive lemurs.[1,14,45] Although these parameters are useful in assessing in some species,[44] recent investigations[13,50] have demonstrat-

Table 6. Enteric parasites identified from *Eulemur fulvus albifrons* from four sites in Madagascar.

Site	No. positive/ no. examined	Species identified[a]
Betampona Special Reserve	1/10	Callistoura, Lemuricola
Marojejy National Park	1/2	Nematode
Masoala National Park	6/7	Lemuricola (6), Lemurostrongylus (4), Nochtia (3), Enterobius (1)
Nosy Mangabe Special Reserve	10/10	Callistoura (8), *Parahabdonema longistrata* (5), *Trichurus lemuris* (4), Lemurostrongylus (4), pinworm larvae (3), Lemuricola (1)

[a] Numbers in parentheses indicates number of individuals in which parasite was detected.

ed that variability between species and individuals makes extrapolation hazardous, and emphasizes the need for species-specific references. The data presented here further emphasize that point, and suggest that location, season, or sample size may also introduce variability.

Differences noted in fat-soluble vitamins are also very likely due to season and sample size. Variation is present in retinols and tocopherols. However, there is a consistent lack of carotenoids in all samples, indicating that lemurs may metabolize or utilize these compounds differently. This is consistent with vitamin analyses from other wild lemur species[10,21–23] but different from most primate species that have detectable carotenoids.[4,43] In fact, most primates are considered carotenoid accumulators, whereas ungulates are more commonly nonaccumulators.[43] This supports the suggestion that vitamin metabolism in prosimians is clearly different than other primates. Differences are also present in the vitamin D precursor 25-hydroxycholecalciferol. Vitamin D precursors come from two sources, either dietary ergocalciferol or from ultraviolet-light conversion of cholecalciferol in the skin.[48] Differences noted between populations is probably a reflection of dietary intake, as populations collected during the same season (BSR and MJNP) have the largest difference.

Fecal bacteria and enteric parasites are reported; however, comparisons between sites were not done. Test results were not quantified, and small numbers of samples and probable seasonal variation make such comparisons unrewarding. Enteric bacteria typically considered to be pathogenic in lemurs (*Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*)[2,3,30,32,41] were not detected. Freezing fecal samples has been validated for preserving viability for most species of bacteria with the exception of *Campylobacter*.[5,15] It is interesting to note the lack of mites on brown lemurs from NMSR. The Laelapidae mite, which is commonly found on several lemur species at multiple sites, has yet to be described to the species level. However, none were found on white-fronted brown lemurs on the island of Nosy Mangabe, suggesting the isolation of the island site may prevent the mite presence. As this mite does not seem to be associated with clinical disease, this isolation may not be significant from a health perspective.

Unfortunately, small and incomplete sample sets make comparison of some test results difficult. Toxoplasmosis titers were only determined for lemurs from two sites (10 individuals); however, 2 individuals from the same site have detectable IgG titers. Toxoplasmosis has been reported in lemur species,[9] often with high mortality. This is the first report of positive titers detected in apparently healthy wild brown lemurs. The definitive host for *Toxoplasma gondii* is felid species; therefore, detection of titers indicates exposure to domestic cats, as there are no native felids in Madagascar. Domestic cats are kept as pets and feral cats are present in many areas. Likewise, fecal samples for *Cryptosporidium* and *Giardia* detection were only available from 10 individuals from two sites. Detection of these pathogens in lemurs would most likely indicate exposure to humans or their associated animals, including livestock, pets, and vermin. All tested fecal samples were negative. Although sample sizes for these three pathogens were too small to make comparisons between sites, they do provide useful information on disease exposure. The identification of human-associated pathogens has been associated with human contact in mountain gorilla (*Gorilla gorilla berengei*), possibly related to ecotourism.[16,25,26]

In conclusion, analysis of health parameters from small sample sets from four sites revealed differences that upon critical evaluation are thought not likely to be clinically significant but rather sample-size artifacts. This supports the recommendation that reference values be collected on larger sample sizes and from a representative cross section of a wild population. Additionally, the evaluation of nutritional parameters must take into account location and season when comparing values, as these variables may affect the results. Differences in parasite populations (high enteric parasite population and lack of ectoparasites at NMSR) may indicate isolation (island population) and increased density.

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