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BASELINE HEALTH AND NUTRITION EVALUATION OF TWO SYMPATRIC NOCTURNAL LEMUR SPECIES (*AVAHI LANIGER* AND *LEPILEMUR MUSTELINUS*) RESIDING NEAR AN ACTIVE MINE SITE AT AMBATOVY, MADAGASCAR

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Abstract: Extractive industries can have significant impacts on ecosystems through loss of habitat, degradation of water quality, and direct impact on floral and faunal biodiversity. When operations are located in sensitive regions with high biodiversity containing endangered or threatened species, it is possible to minimize impact on the environment by developing programs to scientifically monitor the impact on resident flora and fauna species in the early phases of operation so that effects can be mitigated whenever possible. This report presents the baseline health, nutrition, and trace mineral evaluation for 33 *Avahi laniger* (Eastern wooly lemur) and 15 *Lepilemur mustelinus* (greater sportive lemur) captured and given complete health evaluations that included the measurement of fat-soluble vitamins and trace minerals in addition to routine complete blood counts, serum chemistries, and parasite evaluations. All lemurs appeared healthy on physical examination despite the presence of minor wounds consistent with interspecies aggression in some individuals. Serum chemistry values were within expected ranges for other lemur species; however, *A. laniger* erythrocytes were significantly smaller than those of *L. mustelinus.* Serum nickel values were markedly higher than expected in both species, and selenium, copper, and cobalt levels were higher in *L. mustelinus* compared with *A. laniger* at the study site, as well as values for *I. indri* or *P. diadema* reported from other locations. Endoparasites and ectoparasites were typical of those reported in other wild lemur species, but load and diversity varied between *A. laniger* and *L. mustelinus* despite inhabiting the same forest ecosystem. This baseline assessment provides the foundation for ongoing monitoring.

Key words: *Avahi,* Health, *Lepilemur*, Nutrition, Sportive lemur, Wooly lemur.

INTRODUCTION

Madagascar is considered one of the world's top conservation priorities due to its unparalleled levels of species diversity and endemism.[42] Habitat destruction resulted in an estimated 80% reduction of core forests between 1950 and 2000.[23] Deforestation has resulted in fragmentation and degradation of Madagascar's forest ecosystems, where as much as 90% of the island's endemic biodiversity resides.[2,13,23] Continued habitat degradation and other anthropogenic disturbances such as hunting, trapping, and mining may have profound effects on wildlife populations in Madagascar.[1]

The Ambatovy Joint Venture is a nickel and cobalt mine and processing plant located on the east coast of Madagascar. The project includes an 1,800-ha mine site surrounded by a 3,600-ha forest conservation zone located near the town of Moramanga, a 217-km pipeline, and a processing plant and refinery near the city of Tamatave.[54] Ore extraction began in early 2011 and is expected to continue for 27 years.[51] The mine site is located in an area of mid-altitude rain forest that consists of a matrix of zonal, transitional, and azonal rainforest with high biodiversity. To be compliant with International Finance Corporation (IFC) Performance Standards (2012) and the Business and Biodiversity Offset Programme guidelines,[6] the project aims to achieve no net loss of biodiversity, and preferably a net gain, and has developed an Ambatovy Biodiversity Management System (ABMS) in line with this objective. The Lemur Management Plan (LMP), a subsection of the ABMS, specifically addresses the management of the 13 confirmed lemur species

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located within forests surrounding the mine site footprint.

Four species were selected for long-term monitoring as part of the LMP spatial monitoring program based on International Union for the Conservation of Nature (IUCN) designation of threatened status, ecologic and habitat requirements, and the feasibility of capturing a sufficient number of individuals to allow for valid statistical comparisons. Based on these criteria, *Avahi laniger* (VU, vulnerable), *Lepilemur mustelinus* (NT, near threatened), *Indri indri* (CR, critically endangered), and *Propithecus diadema* (CR, critically endangered), were selected for long-term spatial and health monitoring. *A. laniger* is a member of the Indriidae family, whereas *L. mustelinus* is a member of the Lepilemuridae family. Both species are small, folivorous, nocturnal lemurs weighing between 0.8 and 1.4 kg[41] and are sympatric in the forests in the Ambatovy region. Neither species has been extensively studied, and health parameters for free-roaming wild individuals are not available. This paper presents preliminary health evaluation information for *A. laniger* and *L. mustelinus*. Due to differences in diurnal rhythms, body size, feeding ecology, and habitat use, data for *I. indri* and *P. diadema* are presented in a separate report.

MATERIALS AND METHODS

This study was conducted at the Ambatovy Joint Venture mine site located 80 km east of the capital city of Antananarivo, near the town of Moramanga. The site is comprised of a mine foot print of 1,800 ha and an adjacent 3,640-ha tract of forest, the Conservation Zone (CZ), managed by the Project as a conservation area that will not be mined. The site is a mid-altitude forest that consists of a matrix of zonal, transitional and azonal rainforest with high biodiversity. Lemur species documented within the study site include *Microcebus lehilahytsara, Allocebus trichotis, Cheirogaleus crossleyi, Lepilemur mustelinus, Hapalemur griseus griseus, Prolemur simus, Eulemur fulvus, Eulemur rubriventer, Varecia variegata, Avahi laniger, Propithecus diadema, Indri indri,* and *Daubentonia madagascariensis.*[54]

Health evaluations were conducted consistent with previously published protocols for conducting health assessments in wild lemurs.[10,11,25,29–34,48] Activities in this project complied with all US standards for the use of animals in research, and the protocol was approved by Duke University's Institutional Animal Care and Use Committee. In addition, all applicable national research permits were obtained in Madagascar.

Lemurs were individually anesthetized using tiletamine and zolazepam (Telazol®, Fort Dodge Animal Health, Overland Park, Kansas 50501, USA; or Zoletil, Virbac Animal Health, Milperra New South Wales 2214, Australia; 20 mg/kg, im) delivered by dart (Type "C" Disposable Dart, Pneu-Dart, Williamsport, Pennsylvania 17701, USA) or by hand injection for *L. mustelinus* located in sleeping holes. Once anesthetized, each animal was weighed, sex was determined, and age was estimated. Each lemur received a subcutaneous radio transponder chip (Trovan Ltd, Weilerswist 53919, Germany) implanted between the scapulae for permanent identification and either a radio collar or nylon collar and dog tag for subsequent spatial monitoring. Physical examinations consisted of recording the rectal temperature, heart rate, and respiratory rate, evaluating the condition of dentition, and systematically examining all body systems. Blood samples were collected directly into vacutainer tubes containing EDTA or into serum separator clot tubes containing clot activator. Clot activator tubes were centrifuged within 30 min of collection, and the serum was transferred into plastic cryovials and frozen at –20°C until transported (frozen) to the United States. Once in the United States, the samples were stored at –80°C until analysis.

Fecal samples were collected as either freshly voided feces or from the rectum during examination using a fecal loop and placed into transport medium (Remel Company, Lenexa, Kansas 14428, USA). If ectoparasites were present, they were gently removed using thumb forceps and placed into 70% ethyl alcohol for identification.

Following the health examination and sample collection, each animal was given sterile balanced electrolyte solution subcutaneously at the rate of 10 ml/kg and held in secured cloth bags until fully recovered from anesthesia. Lemurs captured in the morning were released at the original capture location the evening of the same day. Lemurs captured in the afternoon or evening were held overnight in a secure location after examination and released at the original capture location the following evening.

Complete blood cell counts (CBCs) were done on site using an automated cell counter (HemaTrue Hematology Analyzer, Heska, Loveland, Colorado 80538, USA). Serum was submitted for a biochemical profile; measurement of the fat-soluble vitamins A, E, beta carotene, and 25, hydroxycholecalciferol; and serum trace mineral analysis for cobalt, copper, iron, nickel, manganese, molybdenum, selenium, and zinc to the

Table 1. Physical examination parameters for adult *L. mustelinus* and *A. laniger* at Ambatovy, Madagascar.[a]

Species	Sex	*n*	Weight (kg)	Body temperature (°C)	Heart rate (beats/min)	Respiratory rate (breaths/min)
L. mustelinus	M	10	1.005	34.3	147	42
			0.85–1.15	31.9–37.8	96–212	15–80
	F	5	1.06	35.6	148	52
			0.86–1.15	33.2–38.1	114–208	30–60
	B	15	1.01	34.8	148	44
			0.85–1.15	31.2–38.2	90–212	15–80
A. laniger	M	13	1.1	35.8	172	56
			0.75–1.5	32.9–38.1	120–236	36–148
	F	20	1.26	35.9	170	54
			0.75–1.5	31.7–38.6	112–222	28–88
	B	33	1.2	35.6	172	54
			0.74–1.5	31.7–38.6	112–236	28–148

[a] Values provided are median and range. B, both; F, female; M, male.

Diagnostic Center for Population and Animal Health (Lansing, Michigan, USA). Fecal samples in transport medium were submitted to the Cornell University Animal Health Diagnostic Center (Ithaca, New York, USA) or Lincoln Memorial University (C. Faulkner) for examination and identification of parasites and ova by standard centrifugation techniques and for detection of *Cryptosporidium* and *Giardia* by ELISA. Ectoparasites were submitted for identification to the Ohio State University (Columbus, Ohio, USA). Statistics were run using NCSS Statistical Software program (NCSS, LLC, Kaysville, Utah 84037, USA). Due to small sample sizes, values are presented as median and range rather than mean and SD. Statistically significant values were determined using a two-sided *t*-test and significance was set at $P = 0.05$.

RESULTS

Four capture sessions were conducted between September 2012 and March 2014. In total, 15 *L. mustelinus* (10 males and 5 females) and 33 *A. laniger* (13 males and 20 females) were captured and examined. One *A. laniger* female was carrying an infant (estimated as less than 1 mo of age) and five *A. laniger* and two *L. mustelinus* were pregnant at the time of capture.

Results for body weight, rectal temperature, heart rate, and respiratory rate are presented in Table 1. No significant differences were observed between males and females of the same species for any of the physical examination parameters examined, and body weights were within published ranges for these species.[41]

Results of hematology and serum chemistries are presented in Tables 2 and 3, respectively. Median values for white blood cells (WBCs), packed cell volume (PCV), and white blood cell differential counts for granulocytes, lymphocytes, and monocytes were consistent with values published in other lemur species for which health assessments are available.[10,11,17,25,29–34,40,47,49] For *A. laniger* and *L. mustelinus,* hematology values for all parameters were similar for the two species with the exception of mean corpuscular volume (MCV) and red cell distribution width (RDW), which are measurements of red blood cell size. Both MCV and RDW are significantly smaller for *A. laniger* than for *L. mustelinus*. The median MCV and RDW for *A. langier* was 39.7 and 25.8 fl compared with 53.9 and 37.3 fl, respectively, for the same measurements in *L. mustelinus*. For serum chemistries, statistically significant differences were identified between the two species for sodium and creatine phosphokinase (CPK). Median sodium values were 143 mmol/L for *L. mustelinus* compared with 149 mmol/L in *A. laniger*. The median CPK value of *L. mustelinus* was substantially higher at 2,174 U/L than in *A. laniger* with a value of 883 U/L.

Information on the species of external parasites present on lemurs by species is provided in Table 4. Both species had low levels of ectoparasites (*A. laniger*, 24%; *L. mustelinus*, 47%). *L. mustelinus* hosted two species of ticks, whereas *A. laniger* was found to have only one species, *Haemaphysalis lemuris*. Similarly, the mite *Liponysella madagascariensis* was found only on *A. laniger*, but *Trichophilopterus babakotophilus* was present on both. Quantitative assessment of ectoparasite loads was not conducted; therefore, it is not possible to comment on differences in the numbers of ectoparasites between the two species.

Table 2. Hematology parameters for *L. mustelinus* and *A. laniger* at Ambatovy, Madagascar.[a]

Parameter	*A. laniger* ($n = 32$)	*L. mustelinus* ($n = 15$)
WBC ($10^3/\mu l$)	7.1	7.7
	2.4–18	3.2–15.3
PCV (%)	44.8	44
	31–56	33–51.5
Granulocytes (%)	49.2	42.6
	17.1–59.2	30.3–75.6
Lymphocytes (%)	44.1	48.5
	18.6–78	20.6–62.1
Monocytes (%)	5.2	9.9
	2–27.7	3.5–31
Hgb (g/L)	14.4	14.6
	9.7–18.4	10.5–16.3
RBC (10^6/L)	9.21	7.77
	6.2–11.9	5.5–9.6
Plt (10^9/L)	277	295
	68–602	216–602
MCV (fL)	39.7[A]	53.9[B]
	25.6–45	47.1–56.4
RDW (fL)	25.8[A]	37.3[B]
	23–37.6	33.2–40.2
MPV (fL)	6.4	7.2
	5.9–8.1	6.6–8.5

[a] Values in rows with different superscripts are significantly different ($P < 0.05$). Values are median and range. Hgb, hemoglobin; MCV, mean corpuscular volume; MPV, mean platelet volume; PCV, packed cell volume; Plt, platelet count; RBC, red blood cell count; RDW, red blood cell width; WBC, white blood cell count.

Table 3. Serum chemistry parameters for *L. mustelinus* and *A. laniger* at Ambatovy, Madagascar.[a]

Parameter	*A. laniger* ($n = 32$)	*L. mustelinus* ($n = 15$)
AST (U/L)	57	26
	13–220	4–189
ALT (U/L)	24	23.5
	5–116	7–55
T Bili (mg/dl)	0.6	0.4
	0.4–3.8	0.3–0.7
ALP (U/L)	71	62.5
	37–197	35–612
TP (g/dl)	6.2	6.4
	5–7	4.7–7.7
Alb (g/dl)	4.1	4.5
	3.5–4.8	3.1–5.3
Glob (g/dl)	1.9	2.1
	1.4–2.9	0.9–3.0
Chol (mg/dl)	139	122
	85–199	71–175
BUN (mg/dl)	11	7
	4–16	2–18
Creat (mg/dl)	0.6	0.5
	0.4–0.8	0.3–0.7
Ph (mg/dl)	3.7	4.4
	1.8–6.9	2–8.1
Ca (mg/dl)	9.9	10
	8.7–10.4	8.6–11.4
Gluc (mg/dl)	166	108
	83–320	67–233
Amylase (U/L)	200	87
	92–328	44–165
Na (mmol/L)	149[A]	143[B]
	146–156	138–151
K (mmol/L)	3.9	5.1
	2.9–4.5	4.1–5.5
Cl (mmol/L)	108	104
	102–115	99–111
CPK (U/L)	883[A]	2174[B]
	151–3444	156–16660
Mg (mg/dl)	2.7	3.2
	1.9–3.4	2.5–4.4

[a] Values are median and range. Values in rows with different superscripts are significantly different ($P < 0.05$). Alb, albumin; ALP, serum alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; Chol, cholesterol; CPK, creatinine phosphokinase; creat, creatinine; Glob, globulin; Gluc, glucose; T bili, total bilirubin; TP, total protein.

Endoparasites identified in lemurs at this site are presented in Table 5 and are consistent with those identified in lemurs at other sites.[8,26,46] It was only possible to collect fecal samples from 30 *A. laniger* and 5 *L. mustelinus*. Strongyles, including *Lemurostrongylus, Parahabronema*, and a nonspeciated variety, were the most commonly identified endoparasite and were more often identified in *L. mustelinus* (80%) than in *A. laniger* (60%). Other nematodes identified to the genus level were *Lemuricola* in two *Lepilemur* and an unspeciated pinworm in one *Lepilemur*. A hymenolepid tapeworm ovum was also reported in one *A. laniger*. The protozoal parasites *Giardia* and *Cryptosporidium* were not detected in any fecal sample.

Results of fat-soluble vitamin analysis are given in Table 6 with values from two other members of the Indriidae family, *I. indri* and *P. diadema*, available in the literature provided for comparison. The only statistically significant difference noted was that 25 hydroxyvitamin D was higher in *A. laniger* than in *L. mustelinus*, with median values of 19 and 16 nmol/L, respectively. Beta carotene levels were below analyzer range for 28 of 30 *A. laniger* and 13 of 14 *L. mustelinus*, indicating that these two species do not accumulate beta carotene to any significant degree.

Values for serum trace minerals are presented in Table 7. Comparative data are not available for wild or captive *A. laniger* or *L. mustelinus*. When comparing the two species in this study, however,

Table 4. Ectoparasites detected in *A. laniger* and *L. mustelinus* at Ambatovy, Madagascar.

Ectoparasite	*A. laniger* ($n = 31$)	*L. mustelinus* ($n = 15$)
Haemaphysalis lemuris	4	5
Ixodes lemuris	0	4
Trichophilopterus babakotophilus	2	2
Liponysella madagascariensis	1	0
Multiple species	1	4
No ectoparasites	25	8

Table 5. Endoparasites detected in *A. laniger* and *L. mustelinus* at Ambatovy, Madagascar.

Endoparasite	*A. laniger* ($n = 30$)	*L. mustelinus* ($n = 5$)
Lemurostrongylus	5	0
Strongyle[a]	13	1
Parahabronema	2	3
Pinworm[a]	0	1
Lemuricola	0	2
Hymenolepid tapeworm[a]	1	0
Ingloxyuris inglisi	0	1
Crypto/Giardia	0	0
Multiple species	0	3
No parasites detected	9	0

[a] Not further identified.

there are significant differences between levels of the trace minerals cobalt, copper, selenium, and zinc. *L. mustelinus* has significantly higher levels of these minerals than *A. laniger*. Differences were most marked for selenium, with *L. mustelinus* having a median value of 181 ng/ml, more than six times that of *A. laniger* at 29 mg/ml. Cobalt levels were three times higher in *L. mustelinus* (median = 12.9 ng/ml) than in *A. laniger* (median = 4.33 ng/ml), whereas the differences in zinc and copper, although significant, were more modest; 2.1 μg/ml in *L. mustelinus* compared with 1.3 μg/ml in *A. laniger* for zinc and 1.0 μg/ml compared with 0.8 μg/ml for copper, respectively. Fifteen of 33 samples from *A. laniger* and 12 of 16 samples from *L. mustelinus* had molybdenum levels below analyzer range, preventing statistical analysis.

DISCUSSION

Long-term studies of habitat degradation and fragmentation demonstrated detrimental effects on lemur biodiversity, including decreased genetic diversity, reproductive success, and general fitness.[7,14,18,24,25,27] Habitat of decreased quality sustains smaller populations of many species and increases a population's susceptibility to stochastic events such as disease outbreaks. This is theorized to be due to animals having impaired immune responses as a result of increased stress from intraspecies aggression secondary to competition for limited resources or from nutritional imbalances adversely impacting immune system function.[1,3,14,25] Increased edge effects also increase exposure to humans and domestic livestock, thereby elevating the risk of anthropozoonotic disease transmission.[26,37,43] Transmission of diseases from humans and livestock to wildlife is particularly a concern to endangered primate species.[19,20,35,43] Bublitz et al.[5] demonstrated that lemurs inhabiting disturbed forests adjacent to Ranomafana National Park with exposure to human activity and domestic livestock harbor multiple strains of enteric pathogenic bacteria including enterotoxigenic *Escherichia coli, Shigella* spp., *Salmonella enterica, Vibrio cholerae,* and *Yersinia* spp. (*enterocolitica* and *pseudotuberculosis*). The same strains of pathogenic bacteria were present in local villagers, domestic livestock, and rats sampled at the same site. Notably, the pathogenic strains of bacteria were not present in the feces of lemurs living in undisturbed forests within the Park. Scabies mites transmitted from humans have been documented to infect free ranging mountain gorillas (*Gorilla gorilla*) in Uganda.[35] Ebola and anthrax have spread between human and gorilla populations in West Africa, causing high mortality rates in both groups.[39] Also, human paramyxovirus has resulted in cyclical mortality in wild chimpanzees (*Pan troglodytes*).[38]

Effects of mining may lead to profound ecologic changes including vegetation loss, soil erosion, and poor water quality[12,21] in habitat adjacent to mine sites. In addition, mining may elevate concentrations of minerals in the air, water, soil, and sediment in regions within close proximity.[12] Evaluating the health effects of anthropogenic disturbance is an important tool for detecting potential impacts on endangered species located in regions associated with active mining, which can then be used to inform appropriate mitigation actions. To determine whether a particular species is being adversely affected by human activities, establishing baseline values from individuals in the area of concern prior to the occurrence of significant changes in the regional habitat is a reasonable approach and allows scientists to monitor changes over time in the populations as habitat is altered.

Table 6. Serum fat-soluble vitamin values for *L. mustelinus* and *A. laniger* at Ambatovy, Madagascar.[a]

Vitamin	*A. laniger* ($n = 30$)	*L. mustelinus* ($n = 14$)	*I. indri*[30]	*P. diadema*[25]
D (nmol/L)	19[A]	16[B]	20[A]	19[A]
	6–42	2–27	9–43	11–46
A (ng/ml)	396[A,B]	251[A]	685[C]	515[B]
	222–1734	229–523	376–3009	156–702
E (μg/ml)	8.03[A]	2.97[A,B]	5.36[B]	9.21[A,B]
	4.84–44.54	1.25–41.67	2.88–22.04	4.03–11.89
Beta carotene (μg/ml)[b]	0.25, 0.035	0.25	0.68	0.7
			0–1.45	0.2–1.6

[a] Values in rows with different superscripts are significantly different ($P < 0.05$).
[e] Beta carotene not statistically analyzed (see text for details).

A. laniger and *L. mustelinus* were selected as important species for monitoring because little is known regarding their ability to adapt to changing habitats. Despite being sympatric, nocturnal, folivorous lemurs with a similar adult body mass of approximately 0.8–1.4 kg, their feeding ecology and habitat use differs. During the day, *L. mustelinus* sleeps in hollow trees or dense vegetation high in the canopy, whereas *A. laniger* prefers to sleep on branches or in dense vegetation at an average height of 3 m.[22,50] Although both consume primarily leaves, *A. laniger* consumes leaves of higher nutritional quality with higher levels of easily extractable protein low in alkaloids, whereas *L. mustelinus* subsists on lower-quality leaves with higher fiber, lower protein content, and higher levels of alkaloids.[16]

Physical examination findings and median values for hematology and standard chemistries for animals in this study were largely normal and consistent with ranges published in other wild lemurs,[8,10,11,17,25,29–34,40,46,47,48] suggesting that most individuals were in good condition despite the presence of minor injuries typical of intraspecies aggression in some individuals. Although median values for Na and CPK between *A. laniger* and *L. mustelinus* were statistically different, the small sample sizes may not be reflective of true population differences. The duration of time necessary to pursue and capture particular individuals could impact both values sufficiently to alter median levels of either group as a whole. In contrast, the differences in RBC size as indicated by marked variation in median values of MCV and RDW between *A. laniger* and *L. mustelinus* would not be expected to be affected by differences in capture stress or short duration alterations of physiologic status. Although the finding should be

Table 7. Serum trace mineral results for *L. mustelinus* and *A. laniger* at Ambatovy, Madagascar.[a]

Mineral	*Avahi* ($n = 33$)	*Lepilemur* ($n = 15$)	*I. indri*[30]	*P diadema*[25]
Co (ng/ml)	4.3[A]	12.9[B]	9.3[B]	3.1[A]
	0.4–29.2	1.5–116.4	3.0–35	0.6–14.1
Cu (μg/ml)	0.8[A]	1.0[B,C]	0.7[A,B]	1.0[C]
	0.4–1.6	0.8–1.7	0.5–2.0	0.7–1.5
Fe (μg/dl)	138[A]	172[A]	253B	306[B]
	44–377	46–355	158–500	69–769
Ni (ng/ml)	9.4[A]	11.7[A]	4.7[B]	NA
	3.3–36.7	5.5–27.9	1.3–8.3	
Mn (ng/ml)	6.7[A]	5.4[A,B]	3.9[B]	4.0[B]
	3.6–39.2	2.9–20.9	2.2–12.5	0.8–17.1
Mo (ng/ml)[b]	0.7	0.7	2.5	1.3
	0.5–1.3	0.5–1	0.5–10.7	0.1–10.5
Se (ng/ml)	29[A]	181[B]	50[C]	19[D]
	9–93	68–246	17–100	10–68
Zn (μg/ml)	1.3[A]	2.1[B]	0.7[C]	0.8[C]
	0.5–2.3	1.4–3.7	0.5–2.1	0.5–1.9

[a] Values are median and range. Values in rows with different superscripts are significantly different ($P < 0.05$).
[b] Molybdenum values were not evaluated statically (see text for details).
[c] NA = not available

verified in populations of *A. laniger* and *L. mustelinus* at other sites, if consistent, the differences in RBC size underscore the importance of determining reference values for each species individually.

Endoparasites were typical of those reported for lemurs elsewhere.[26] No *Giardia* or *Cryptosporidium* were detected in lemurs in this study. *Giardia* has been reported in bamboo lemurs (*Hapalemur griseus*) in degraded areas in Ranomafana National Park.[49] The authors suggested that exposure of lemurs to humans or livestock was the likely cause. There is no evidence of such anthropogenic introduction of these parasites at the study site at the time animals were sampled for this project; however, further encroachment of domestic species and people into the forests as mining proceeds could result in the introduction of parasitic pathogens to the detriment of lemurs in the region.

The fat-soluble vitamins A, D, E, and K and beta carotene, a precursor to vitamin A, are a measure of nutritional status as their levels are less labile than the B complex vitamins and vitamin C, which are water soluble. Fat-soluble vitamins are typically acquired from food sources; however, vitamin D may also be generated in the skin secondary to ultraviolet exposure from sunlight. As with many other parameters, reference values for the two species studied here are not available. Therefore, values for *I. indri* and *P. diadema,* both diurnal members of the same family as *A. laniger,* are provided for comparison.

Animal species that have detectable beta carotene levels are considered carotenoid-accumulating species,[53] whereas species that do not typically have detectable beta carotene are considered "carotenoid nonaccumulators." Anthropoid primates in captivity are reported to have detectable levels of beta carotene.[9] Beta carotene was not detectable in measurable amounts in either *A. laniger* or *L. mustelinus* in this study. Several other lemur species including red ruffed lemurs (*Varecia rubra*), black and white ruffed lemurs (*Varecia variegata variegata*), white fronted brown lemurs (*Eulemur albifrons*), red fronted brown lemurs (*Eulemur rufus*), black lemurs (*Eulemur macaco*), Verreaux's sifaka (*Propithecus verreauxi*), and Decken's sifaka (*Propithecus deckeni*) do not accumulate carotenoids and therefore represent some of the very few nonaccumulator primate species.[10,11,29,30,31,33]

Vitamin D is generated via two metabolic pathways: exogenously via consumption of foods containing vitamin D or from conversion in the skin after exposure to ultraviolet radiation from sunlight. In primates, both pathways are used; however, the degree to which a given species depends on the different pathways is still unclear. Comparison of vitamin D levels reveals that nocturnal *L. mustelinus* has significantly lower values than diurnal *I. indri*[30] and *P. diadema*.[25] Additionally *L. mustelinus*, which sleeps in tree holes during the day, has statistically lower levels than *A. laniger*, which sleeps in trees during the day.[25,29] This may indicate that exposure to natural sunlight contributes to vitamin D generation in both diurnal and nocturnal lemur species.

Minerals that are integral to tissue structure and organ function (calcium, phosphorus, potassium, sodium, chloride) are termed macro-minerals, whereas trace minerals are present in lower levels and function as cofactors in enzymatic pathways and physiologic functions.[4] Deficiencies in trace minerals often manifest in subtle but physiologically important ways, such as altered metabolic pathways and altered immune responses. No comparative data for serum trace minerals are available for the species studied in this report. Published values for *P. diadema*[25] and *I. indri*[29] are provided for comparative purposes.

Although *A. laniger* and *L. mustelinus* at Ambatovy coexist sympatrically in the same forests, cobalt, copper, selenium, and zinc were all higher in *L. mustelinus* than in *A. laniger*, with differences in the serum content of cobalt and selenium being particularly marked. Median levels of selenium and cobalt in *L. mustelinus* far exceeded those of either *A. laniger* at Ambatovy, *I. indri* evaluated at Analamazoatra, or *P. diadema* at Tsinjoarivo.

In domestic animals, blood selenium levels are considered adequate when above 100 ng/ml, whereas levels $<$50 ng/ml are considered deficient. Chronic toxicity has been reported when blood levels exceed 1 ppm or 1,000 ng/ml.[36] By these standards, *A. laniger* at Ambatovy, as well as *I. indri* evaluated at Analamazoatra by Junge and Barrett,[29] would be considered selenium deficient, whereas *L. mustelinus* falls within acceptable normal ranges.

Cobalt, in the form of hydroxycobalamine, is a vital component of vitamin B_{12}. In excess, cobalt is toxic; however, only in rare circumstances does it reach levels of concern. In humans, toxicity becomes apparent at concentrations $>$300 ng/ml.[15,52] Although serum levels of cobalt are significantly different between *A. laniger* and *L. mustelinus* at Ambatovy, levels are well below toxic levels. Cobalt levels in *A. laniger* are similar to those in *P. diadema* at Tsinjoarivo, a site distant from Ambatovy. Although *A. laniger* and *L. mustelinus* inhabit the same forests in close proximity to mine

activities extracting cobalt from the soil, the difference in species levels of the element suggests that dietary differences, rather than mine operations per se, are the cause of the differences observed. Plants can accumulate a variety of trace minerals including selenium, cobalt, and nickel from the soil, and plant species vary significantly in their ability to concentrate the element.[44] Given that *A. laniger* and *L. mustelinus* have different foraging strategies, it is possible that variation in blood cobalt and selenium levels in the two lemur species at Ambatovy reflects differences in the mineral content of plants consumed. Without comparisons from *A. laniger* at other locations, it is not possible to determine whether the low selenium levels observed in *A. laniger* at Ambatovy is normal for the species or is specific to this particular site. Similarly, comparisons of plants consumed by *A. laniger* and *L. mustelinus* at Ambatovy, as well as changes of plant ecosystems secondary to mining activity, would be needed to evaluate whether anthropogenic changes at the site are impacting trace mineral status in the two species.

In comparing trace mineral levels of *A. laniger* and *L. mustelinus* at Ambatovy with *I. indri* and *P. diadema* sampled at other sites, notable differences include markedly lower levels of iron and higher levels of nickel and zinc in *A. laniger* and *L. mustelinus* in this study. The fact that nickel values of *A. laniger* and *L. mustelinus* are high at the site may be related to the presence of ultramafic soils at the site containing high levels of nickel, and hence, the reason for situating a nickel mine at the site where this study was undertaken. Possible causes for the results observed include the ingestion of hyperaccumulator plant species that concentrate these minerals[28] or differing feeding strategies between *A. laniger* and *L. mustelinus* such that the two species preferentially select plant parts or species higher in nickel and zinc than do *I. indri* or *P. diadema*.[44] Animals in this study could also develop high serum levels through the practice of geophagy. *I. indri* and *P. diadema* are known to engage in geophagy as a normal part of feeding behavior.[45] If *A. laniger* and *L.mustelinus* do likewise, high soil levels of trace minerals present in the region would result in high serum levels in the animals tested. Last, it cannot be ruled out that mine operations directly contribute to increased exposure to nickel and zinc through the generation of large amounts of dust, which settles on the leaves of plants at the forest edge. If lemurs at Ambatovy consume foliage or plant parts containing even minimal amounts of dust, prolonged consumption of nickel, zinc or other trace minerals present in the soil could account for the higher serum values of select trace elements in lemurs at Ambatovy.

Extractive industries create significant effects on the environment, which in turn may have significant effects on flora and fauna.[7] Comprehensive evaluation of animal populations before, during, and after extractive activities is necessary for documenting the effects of such projects on biodiversity and to guide management decisions. Preliminary biomedical health evaluations on the lemur populations located within the forests surrounding the Ambatovy Joint Venture mine site did not show obvious signs of ill health despite differences between the two species in levels of vitamin D and the trace minerals copper, cobalt, selenium, and zinc. Levels of the trace minerals evaluated were within normal physiologic ranges for other mammals and humans, and evidence of glaring excess or deficiency of any element was not identified. Sampling was done during the early phases of mine operations, and thus any potential impacts may be too early to detect via the methods used in this study. Repeated evaluation of both populations at the site as operations expand may be needed to determine whether the differences noted are related to changes in habitat associated with mining or are instead, due to species differences in food choice and habitat utilization. Combining comprehensive longitudinal biomedical evaluations for species of concern with regular, periodic population density monitoring and feeding ecology studies would create a more complete understanding of the impacts that anthropogenic disturbances such as mining have on lemur populations in Madagascar. Such an approach has the potential for identifying impacts early and guiding management decisions and mitigation strategies should adverse effects be identified.

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