American Journal of Primatology 73:632–642 (2011)

RESEARCH ARTICLE

Effects of Anthropogenic Disturbance on Indri (*Indri indri*) Health in Madagascar

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Anthropogenic habitat disturbance impairs ecosystem health by fragmenting forested areas, introducing environmental contamination, and reducing the quality of habitat resources. The effect of this disturbance on wildlife health is of particular concern in Madagascar, one of the world's biodiversity hotspots, where anthropogenic pressures on the environment remain high. Despite the conservation importance of threatened lemur populations in Madagascar, few data exist on the effects of anthropogenic disturbance on lemur health. To examine these impacts, indri (*Indri indri*) populations were evaluated from two forest reserves that differ in their exposure to anthropogenic disturbance. We compared the health status of 36 indri individuals from two sites: one population from a protected, undisturbed area of lowland evergreen humid forest and the other population from a reserve exposed to frequent tourism and forest degradation. Comparison of indri health parameters between sites suggests an impact of anthropogenic disturbance, including significant differences in leukocyte count and differential, 12 serum parameters, 6 trace minerals, and a higher diversity of parasites, with a significant difference in the presence of the louse, *Trichophilopterus babakotophilu*s. These data suggest that indri living in disturbed forests may experience physiological changes and increased susceptibility to parasitism, which may ultimately impair reproductive success and survival. Am. J. Primatol. 73:632–642, 2011.

Key words: Madagascar; *Indri indri*; lemur health; nutrition; wildlife health monitoring; conservation

INTRODUCTION

Madagascar is considered one of the world's top conservation priorities owing to its unparalleled levels of diversity and endemism [Myers et al., 2000]. Intense pressure from habitat destruction and natural resource extraction have contributed to the nearly 80% reduction of core forests between 1950 and 2000 [Harper et al., 2007]. This deforestation has resulted in the fragmentation and degradation of Madagascar's forest habitat, where as much as 90% of its endemic biodiversity resides [Allnutt et al., 2008; Dufils, 2003; Elmqvist et al., 2007; Harper et al., 2007]. This habitat degradation and other anthropogenic disturbance, such as tourism and mining, have profound effects on wildlife populations in Madagascar. Madagascar's flagship wildlife species, the lemurs (Lemuriformes), are particularly vulnerable to anthropogenic habitat disturbance. Here, we explore these impacts on an endangered lemur species, *Indri indri*, by comparing the physiological parameters and parasite diversity of two indri populations in sites under differing levels of anthropogenic disturbance.

Evaluation of the effects of anthropogenic disturbance, including habitat fragmentation, degradation, mining, and other human contact, on wildlife health and nutrition is critical for the management and preservation of endangered species [Acevedo-Whitehouse & Duffus, 2009; Smith et al., 2009; Woodroffe, 1999]. Disease and contamination may directly affect the survival of endangered host populations by reducing physical fitness or indirectly by impairing reproductive success, suppressing population size, or reducing resilience, all of which ultimately can regulate host populations [Cleaveland et al., 2002; Hochachka & Dhondt, 2000].

Monitoring serves to assess nutritional and health status, evaluate the presence of environmental contaminants, identify disease risks, and alert managers to fluctuations in health parameters from baseline levels. The majority of primate pathogens will exert long-term, sublethal effects that can reduce population sustainability [Goldberg et al.,

Contract grant sponsors: St. Louis Zoo Field Conservation; National Science Foundation.

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Received 2 November 2010; revised 31 January 2011; revision accepted 31 January 2011

DOI 10.1002/ajp.20938
Published online 22 February 2011 in Wiley Online Library (wileyonlinelibrary.com).

2008a], and the only way to document these changes is with consistent health monitoring. Health assessments have been completed on a number of lemur species [Clough, 2010; Dutton et al., 2003, 2008; Garell & Meyers, 1995; Irwin et al., 2010; Junge & Garrell, 1995; Junge & Louis, 2002, 2005a,b, 2007; Junge et al., 2008; Miller et al., 2007; Raharivololona, 2006; Raharivololona & Ganzhorn, 2009, 2010; Rainwater et al., 2009; Wright et al., 2009]. However, when compared with other primates, there is a need for more data on the impacts of human disturbance on lemur health as well as an expanded inventory of lemur parasites [Irwin & Raharison, 2009; Raharivololona & Ganzhorn, 2010]. Indri are threatened primarily from habitat loss and fragmentation as a result of agriculture, logging, hunting, and mining within Madagascar [Britt et al., 2002]. The largest of the extant lemur species (5–7 kg), indri are diurnal, folivorous primates found in the low to mid-altitude rainforests of eastern Madagascar [Mittermeier et al., 2006; Powzyk & Mowry, 2003]. This fragmented nature of indri subpopulations, as well as the lack of a thriving captive population, present serious conservation challenges for this species [Britt et al., 2002]. Indri are classified as endangered by the IUCN Red List [Andrainarivo et al., 2008].

Populations of lemurs in disturbed habitats show compromised physiological parameters, indicative of reduced health [Irwin et al., 2010]; yet, more research is needed on the effects of anthropogenic disturbance on lemur health [Irwin et al., 2010]. In order to assess the potential health effects of habitat fragmentation, mining, and anthropogenic exposure on indri, we compared indri populations in disturbed and undisturbed forest reserves in Madagascar that differed in their exposure to humans. The study, presented here, advances knowledge on the effects of environmental change and anthropogenic exposure on patterns of both indri and lemur health and parasitism as a whole [Irwin & Raharison, 2009; Irwin et al., 2010; Junge & Sauther, 2007; Raharivololona & Ganzhorn, 2009; Wright et al., 2009].

METHODS

Study Areas

Indri populations were evaluated from two sites that varied in their disturbance level: Betampona Strict Nature Reserve (BSNR; S17.931389; E49.20333) and forest fragments within a complex of forests we will refer to as the Analamazaotra Forest complex (AFC; S18.93145; E48.41026). Both studies occurred in 2009, from May 23 to June 2 for BSNR and from October 14 to 21 for AFC.

BSNR consists of 2,228 ha of relatively pristine low-altitude evergreen humid forest, surrounded by villages and agricultural areas; access into the reserve is granted by special permit only. BSNR was first created in 1927 and received the most protected natural reserve designation, Strict Nature Reserve, in 1966 [Britt et al., 2002]. Consistent reserve monitoring and environmental education around the reserve have been conducted by the Madagascar Fauna Group since 1990 and improve conservation outcomes in the area. Previous studies estimated a population of 77–147 indri living within BSNR [Glessner & Britt, 2005].

The AFC areas consist of fragmented mid-altitude evergreen humid forest, including the protected areas of Analamazaotra Special Reserve (810 ha), which borders the village of Andasibe, the Analamazaotra Forest Station (700 ha), which is privately managed by the Mitsinjo Association, and Torotorofotsy, a separate conservation area (9,900 ha). Analamazaotra Special Reserve and the Forest Station experience high tourist visitation owing to their convenient location to the capital city of Antananarivo (combined, up to 29,000 foreign tourists per year [R. Dolch, personal communication]). Analamazaotra Forest Station primarily consists of secondary growth forest, which sustains an estimated population of 21–32 indri in at least seven groups. These animals are habituated to humans and have frequent close interactions with guides and visitors. Torotorofotsy, also a mix of primary and secondary forest, has recently been preserved after the discovery of *Prolemur simus*, one of the world's top 25 most endangered primates, within its boundaries [Konstant et al., 2005]. Three kilometers from this site, a large lateritic nickel mining project is under construction, with an annual capacity of 60,000 tons of nickel and 5,600 tons of cobalt per year. Construction on the slurry pipeline began in 2007, and production to begin in 2011 and continue for approximately 27 years [Dickinson & Berner, 2010].

Sample Collection

We conducted health evaluations on indri as part of the ongoing Prosimian Biomedical Survey Project, a project that has assessed more than 550 lemurs of 31 species within 17 sites, since 2000 [Dutton et al., 2003, 2008; Irwin et al., 2010; Junge & Garrell, 1995; Junge & Louis, 2002, 2005a,b, 2007; Junge et al., 2008]. This project is structured to provide collaboration between field biologists and veterinarians involved in conservation projects throughout Madagascar. Veterinarians provide basic medical assistance as needed, and collect standard biomedical samples and health information from animals anesthetized or captured for other purposes. Activities in this project complied with protocols approved by the St. Louis Zoo and Duke University's Institutional Animal Care and Use Committee, as well as adhered to all research requirements in Madagascar and to the American Society of Primatologists principles for the ethical treatment of primates.

Thirty-six indri (20 at BSNR, 16 at AFC) were individually anesthetized using tiletamine and zolazepam (Fort Dodge Animal Health, Overland Park, KS; 15 mg/kg, i.m.) by dart (Type "C" Disposable Dart, Pneu-Dart, Williamsport, PA). 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 given subcutaneous balanced electrolyte solution (Lactated Ringer's Solution, Hospira Inc., Lake Forest, IL) equivalent to the amount of blood collected. Animals were held in cloth bags until fully recovered from anesthesia, and then released at the site of capture.

Blood samples were collected not exceeding 1% of body weight (1 ml/100 g body weight). Whole blood (1/2 ml) was immediately transferred into EDTA anticoagulant, and the remaining volume into non-anticoagulant 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, 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 was placed into a transport medium (Remel Co., Lenexa, KS) for examination of parasite ova. If sufficient feces were obtained, a second 1 cc sample was frozen in liquid nitrogen for bacterial culture. Freezing fecal samples has been validated for preserving viability for most species of bacteria, with the exception of *Campylobacter* [Guder et al., 1996]. If external parasites were discovered on physical examination, they were removed with a cotton swab or forceps and placed into 95% ethyl alcohol.

Laboratory Procedures

Within 8 hr of collection, two blood smear slides were made from each anticoagulant sample, and fixed and stained. A total white blood cell (WBC) count was done within 8 hr of collection (Unipette System, Becton Dickenson Co., Franklin Lake, NJ). 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, MO); toxoplasmosis titer (University of Tennessee Comparative Parasitology Service, Knoxville, TN); fat-soluble vitamin analysis (A, E, carotene, and 25-hydroxycholecalciferol) and trace mineral analysis (Animal Disease Diagnostic Laboratory, Lansing, MI); iron metabolism analysis (Kansas State University, Manhattan, KS), and viral serology (herpesvirus SA8, simian retrovirus 2, simian T-lymphotropic virus, simian immunodeficiency virus, simian foamy virus, measles; Diagnostic Laboratory, Washington National Primate Research Center, Seattle, WA).

Fecal samples in transport medium were submitted for examination for parasites and ova by standard centrifugation techniques and for *Cryptosporidium* and *Giardia* by ELISA (Cornell University Animal Health Diagnostic Center, Ithaca, NY). Fecal cultures were submitted by thawing the frozen fecal samples and inoculating a culture transport swab (Copan Diagnostics, Corona, CA). These swabs were submitted for aerobic culture, specifically requesting *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* identification. 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 *Salmonella* and *Shigella*. After incubation in selenite broth, samples were replated on XLD agar, HE agar, SS agar, Brilliant Green agar, and MacConkey agar. Although freezing does not maintain *Campylobacter* viability well, it was still specifically cultured. Although absence of *Campylobacter* cannot be considered significant owing to this transport issue, presence would be significant owing to its pathogenic potential. Ectoparasites were submitted for identification (Ohio State University, Columbus, OH).

Statistical Analyses

For all numeric parameters, mean $\pm$ SD values of the raw measurements are reported. No values from captive animals were available for comparison [ISIS, 2002]. Before further analysis, all nonbinomial variables were log transformed to address issues of normality. Continuous parameters were then examined for significant differences between males and females and between sites (BSNR vs. AFC) with nonparametric Wilcoxon rank sum tests ($P < 0.05$). Categorical parasite data were examined for statistical differences between sites with contingency tables and Fisher's exact test, which is more appropriate for small sample sizes ($P < 0.05$).

RESULTS

Thirty-six individuals were examined: 20 animals (8 males, 12 females) from the undisturbed site, BSNR, in May 2009, and 16 animals (6 males, 10 females) from the disturbed site, AFC, in October 2009. Of the 36 total individuals, 2 at BSNR were juveniles; we excluded the juvenile animals from mean weight calculation. With nonparametric Wilcoxon rank sum tests, no significant differences existed between males and females for any parameter or between sites for physical exam parameters (Table I). One adult male from AFC exhibited bilaterally symmetrical areas of alopecia on the thighs, with

substantial dermal thickening. No etiologic agent was identified on skin scrapings.

Indri from the two sites differed across a number of complete blood cell counts (Table II). The AFC indri population exhibited higher values for total WBC count, segmented neutrophil, and lymphocyte count, but no difference was found between sites for monocyte or eosinophil count. Significant differences between sites also existed in serum biochemical profiles (Table III). Indri at AFC demonstrated higher values of alanine aminotransferase (ALT), serum alkaline phosphatase (SAP), phosphorus, and magnesium, whereas indri at BSNR exhibited higher values for total protein, albumin, globulin, creatinine, calcium, chloride, and creatine phosphokinase. *Plasmodium* was identified in one indri from AFC (Escalante, personal communication). No differences existed between males and females for any blood cell count or serum chemistry parameter.

TABLE I. Physical Examination Parameters for Indri From Undisturbed (Betampona Strict Nature Reserve) and Disturbed (Analamazaotra Forest Complex) Sites in Madagascar (Mean Values ± SD)

	BSNR (undisturbed) ($N = 20$)	AFC (disturbed) ($N = 16$)	Prob > Z
Weight (kg)	6.41 ± 1.20[a]	6.28 ± 1.07	0.20
Temperature (°C)	36.8 ± 1.0	37.1 ± 0.9	0.91
Pulse (per minute)	95 ± 25	93 ± 17	0.16
Respirations (per minute)	50 ± 12	52 ± 13	0.62

[a]Two individuals at BSNR were juveniles and removed from weight calculations.

TABLE II. Complete White Blood Cell Count and Differential Count Values for Indri From Undisturbed (Betampona Strict Nature Reserve) and Disturbed (Analamazaotra Forest Complex) Sites in Madagascar (Mean Values ± SD)

	BSNR (undisturbed) ($N = 20$)	AFC (disturbed) ($N = 16$)	Prob > Z
White blood cells (per μl)	**4,355 ± 2,726**	**7,264 ± 3,831**	$P < 0.01$
Hematocrit (%)	41.6 ± 6.2	46.3 ± 1	0.165
Lymphocytes (per μl)	**1,961 ± 1,612**	**3,422 ± 887**	$P < 0.01$
Segmented neutrophils (per μl)	**2,415 ± 1,408**	**3,837 ± 1,829**	$P < 0.05$
Eosinophils (per μl)	38 ± 28	117 ± 0	0.3771
Monocytes (per μl)	40 ± 21	653 ± 566	0.105

Bold entries indicate statistically significant values ($P < 0.05$).

TABLE III. Serum Biochemical Profile Values for Indri From Undisturbed (Betampona Strict Nature Reserve) and Disturbed (Analamazaotra Forest Complex) Sites in Madagascar (Mean Values ± SD)

	BSNR (undisturbed) ($N = 20$)	AFC (disturbed) ($N = 16$)	Prob > Z
Total protein (g/dl)	**7.5 ± 0.6**	**6.2 ± 0.9**	$P < 0.001$
Creatinine phosphokinase (IU/l)	**981 ± 408**	**294 ± 328**	$P < 0.001$
Magnesium (mg/dl)	**1.7 ± 1.2**	**3.4 ± 0.8**	$P < 0.001$
Albumin (g/dl)	**5.4 ± 0.3**	**4.7 ± 0.6**	$P < 0.01$
Globulin (g/dl)	**2.1 ± 0.4**	**1.6 ± 0.4**	$P < 0.01$
Creatinine (mg/dl)	**1.5 ± 0.2**	**1.3 ± 0.2**	$P < 0.01$
Calcium (mg/dl)	**9.9 ± 0.6**	**9.0 ± 1.1**	$P < 0.01$
Alanine aminotransferase (IU/l)	**25.9 ± 13.9**	**32.2 ± 10.6**	$P < 0.05$
Serum alkaline phosphatase (IU/l)	**82.0 ± 92.4**	**167.9 ± 161.5**	$P < 0.05$
Phosphorus (mg/dl)	**3.8 ± 1.0**	**4.7 ± 1.9**	$P < 0.05$
Chloride (mEq/l)	**119.2 ± 3.8**	**111.6 ± 14.7**	$P < 0.05$
Sodium (mEq/l)	146.2 ± 2.7	150.4 ± 20.4	0.947
Blood urea nitrogen (mg/dl)	9.3 ± 2.3	9.2 ± 2.9	0.907
Glucose (mg/dl)	106.0 ± 25.8	99.3 ± 19.7	0.456
Total bilirubin (mg/dl)	0.33 ± 0.06	0.36 ± 0.1	0.408
Aspartate aminotransferase (IU/l)	25.4 ± 18.0	35.9 ± 33.4	0.371
Gamma glutamyltransferase (IU/l)	18.1 ± 5.3	16.1 ± 5.4	0.266
Potassium (mEq/l)	3.9 ± 0.5	3.7 ± 0.7	0.114

Bold entries indicate statistically significant values ($P < 0.05$).

Serum trace minerals also differed between sites (Tables IV and V). Indri at AFC exhibited higher values for nickel, cobalt, manganese, and zinc, with differences being at least two-fold greater for all values except zinc. BSNR individuals demonstrated higher values for molybdenum and selenium. The only fat-soluble vitamin exhibiting a site difference was total vitamin A (Table V). We found no significant differences between males and females for any parameter.

Results from fecal parasite exams are described here and in Table VI. Numbers in parentheses indicate the number of positive occurrences and the prevalence within the population tested. In fecal exams from individuals at BSNR, all individuals were positive for *Lemurostongylus* sp. (nine positive,

TABLE IV. Serum Trace Minerals and Iron Analytes for Indri From Undisturbed (Betampona Strict Nature Reserve) and Disturbed (Analamazaotra Forest Complex) Sites in Madagascar (Mean Values ± SD)

	BSNR (undisturbed) ($N = 20$)	AFC (disturbed) ($N = 16$)	Prob > Z
Nickel (ng/ml)	**2.24 ± 0.93**[a]	**5.43 ± 1.15**	$P < 0.0001$
Cobalt (ng/ml)	**6.31 ± 3.37**	**14.65 ± 7.90**	$P < 0.0001$
Manganese (ng/ml)	**3.21 ± 1.22**	**7.55 ± 2.22**	$P < 0.0001$
Zinc (μg/dl)	**0.65 ± 0.08**	**0.83 ± 0.21**	$P < 0.001$
Molybdenum (ng/ml)	**3.85 ± 2.53**	**2.00 ± 2.22**	$P < 0.01$
Selenium (ng/ml)	**66.95 ± 22.39**	**39.88 ± 15.13**	$P < 0.01$
Copper (μg/dl)	0.73 ± 0.36	0.82 ± 0.26	0.116
Ferritin (ng/ml)	102.45 ± 927.13	93.39 ± 34.23	0.960
Transferrin saturation (%)	53.8 ± 15.7	52.67 ± 18.14	0.716
Iron (μg/dl)	254.20 ± 81.60	240.13 ± 84.88	0.631
Total iron-binding capacity (μg/dl)	447.15 ± 82.70	428.75 ± 42.89	0.120

Bold entries indicate statistically significant values ($P < 0.05$).
[a]($N = 10$).

TABLE V. Fat Soluble Vitamins for Indri From Undisturbed (Betampona Strict Nature Reserve) and Disturbed (Analamazaotra Forest Complex) Sites in Madagascar (Mean Values ± SD)

	BSNR (undisturbed) ($N = 20$)	AFC (disturbed) ($N = 16$)	Prob > Z
Total vitamin A (μg/dl)	**696.50 ± 557.29**	**837.75 ± 148.14**	$P < 0.001$
Beta carotene (μg/dl)	1.98 ± 4.34	0.0 ± 0.0	NA
Total vitamin E (μg/dl)	6.15 ± 4.02	5.78 ± 1.53	0.667
25- hydroxycholecalciferol (ng/dl)	20.50 ± 9.96	19.63 ± 4.96	0.665

Bold entries indicate statistically significant values ($P < 0.05$).

TABLE VI. Parasites Documented in Indri From Undisturbed (Betampona Strict Nature Reserve) and Disturbed (Analamazaotra Forest Complex) Sites in Madagascar, Including Their Mode of Transmission, Prevalence (Number Positive/Number Sampled), and *P*-Values as Determined With Fisher's Exact Test

Parasite species	Transmission mode	Prevalence BSNR (undisturbed)	Prevalence AFC (disturbed)	Prob > F
Ectoparasites (total)		2/20 (10%)	6/16 (37.5%)	–
Liponyssella madagascariensis	Direct contact	2/20 (10%)	1/16 (6.3%)	0.852
Trichophilopterus babakotophilus	Direct contact	0/20 (0%)	6/16 (37.5%)	$P < 0.005$
Haemaphysalis lemuris	Direct contact	0/20 (0%)	1/16 (6.3%)	0.46
Ixodes	Direct contact	0/20 (0%)	1/16 (6.3%)	0.46
Endoparasites (total)		9/9 (100%)	10/12 (83.3%)	
Lemurostrongylus sp.	Ingestion	9/9 (100%)	10/12 (83.3%)	0.50
Bertiella sp.	Ingestion	0/9 (0%)	4/12 (33.3%)	0.10
Bertiella-Lemurostrongylus coinfection	Ingestion	0/9 (0%)	4/12 (33.3%)	0.10
Prevalence across all parasites	–	9/20 (45.0%)	11/16 (68.8%)	0.070
Total parasite diversity	–	2	7	

Prevalence across all parasite species was compared between sites using Wilcoxon rank test.

100% prevalence). Of the parasite exams from AFC, we documented *Lemurostongylus* sp. (ten, 83.3%) and *Bertiella* (four, 33.3%); all those infected with *Bertiella* were also coinfected with *Lemurostongylus*. No individuals from BSNR exhibited infection with *Bertiella* (zero, 0%), and samples from both sites were negative for *Giardia* and *Cryptosporidium* (zero, 0%). We did not find BSNR and AFC to be significantly different for the presence of *Lemurostrongylus* ($P = 1.0$), *Bertiella* ($P = 0.102$), or endoparasites as a whole ($P = 1.0$). Cultures from BSNR produced *Enterobacter agglomerans* (now known as *Pantoea agglomerans*) (one, 11.1%), *Escherichia coli* (two, 22.2%), or no growth (six, 66.7%). Cultures from AFC produced *E. coli* (one, 33.3%) or no growth (two, 66.7%) (Table VI).

Visual examinations revealed a number of ectoparasites, with ectoparasite diversity and coinfections higher at AFC (Table VI). At BSNR, mites (*Liponyssella madagascariensis*) were present on two indri (two positive, 10% prevalence). Six indri at AFC exhibited ectoparasites (six, 37.5%), including mites (*L. madagascariensis*, one, 6.3%), lice (*Trichophilopterus babakotophilus*, six, 37.5%), and two types of tick, *Haemaphysalis lemuris* (one, 6.3%) and *Ixodes* (one, 6.3%). Two individuals at AFC had coinfections; one with *Ixodes*, *L. madagascariensis,* and *T. babakotophilus* and another with *H. lemuris* and *T. babakotophilus*. We observed mites frequently not only 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 condition. Results did not indicate significant differences between sites for *L. madagascariensis* (Fisher's Exact Test*:* $P = 0.852$, $N = 35$), *H. lemuris* ($P = 0.457$, $N = 35$), *Ixodes* ($P = 0.457$, $N = 35$), or ectoparasite presence when pooled ($P = 0.068$, $N = 35$). However, we determined that *T. babakotophilus* presence was significantly higher at AFC ($P < 0.01$, $N = 35$).

Serologic assessment for antibodies to the protozoan parasite *Toxoplasma gondii* and viral diseases (herpesvirus SA8, simian retrovirus 2, simian T-lymphotropic virus, simian immunodeficiency virus, simian foamy virus, and measles) were negative for all animals for all diseases tested.

DISCUSSION

This study suggests that indri living in disturbed habitats exhibited physiological changes as compared with indri in a pristine forest. The indri at BSNR inhabit relatively undisturbed primary forest with little human contact, whereas indri at AFC exist within smaller fragments of secondary forest undergoing higher human exposure. These sites also represent two forest types (mid-altitude and low-altitude evergreen humid forest); therefore, it is possible that some of the changes were related to those differences. The division between low- and mid-altitude evergreen humid forests is artificial and a continual gradation exists [Du Puy & Moat, 2003]; therefore, we do not feel that altitude change has a significant effect on disease ecology.

Degraded habitats have demonstrated detrimental effects on biodiversity, including a reduction of species richness, abundance, distribution, genetic diversity, reproductive success, and general fitness through a variety of mechanisms [Chapman et al., 2000; Fahrig, 2003; Irwin et al., 2010; Keesing et al., 2010]. Decreased habitat nutritional quality can impair wildlife fitness, thereby sustaining smaller populations, impairing the immune response, and increasing susceptibility to stochastic events, such as disease outbreaks [Beck & Levander, 2000; Fahrig, 2003; Irwin et al., 2010]. Similarly, a higher frequency of multiple parasite infections as well as higher parasite prevalence occurred in primate populations from edge habitats when compared with interior groups [Chapman et al., 2006a]. This may occur as a result of lowered fitness and immunocompetence or by increased exposure to parasites from other sources owing to edge effects.

Human-mediated introduction of novel parasites, known as ''pathogen pollution,'' poses a serious threat to wildlife health and conservation [Daszak et al., 2000]. Studies have documented that the introduced rodent, *Rattus rattus*, has transmitted diseases to wildlife populations, especially in disturbed habitats where they thrive [Lehtonen et al., 2001]. *R. rattus* remains the primary reservoir for endemic plague (*Yersinia pestis*) in Madagascar [Duplantier & Duchemin, 2003]. Additional health concerns result from other types of anthropogenic disturbance, such as agriculture and mining. Both these activities are widespread throughout Madagascar [Smith et al., 2007]. Long-term effects of mining activities have led to severe ecological changes around mining sites, including vegetation loss, soil erosion, and contamination of rivers [Eisler, 1998; Hammond et al., 2007].

Cobalt and nickel values were more than twofold greater at AFC than at BSNR. Although cobalt values for several lemur species have been recorded [Dutton et al., 2008; Irwin et al., 2010; Junge et al., 2008], nickel values have not been determined for other lemur species, except as measured in hair of *Lemur catta* by Rainwater et al. [2009]. Elevated levels of cobalt and nickel in indri at AFC indicate probable higher levels at that site. Although we did not analyze soil samples for these sites, we can assume that nickel and cobalt levels are elevated in the area owing to the selection of this area for mining. More data will be needed to determine if cobalt and nickel affect health in indri at AFC; however, metal contamination remains an important concern for wildlife health in general. Chronic exposure to metals can exert a health impact [Eisler,

1998; Smith et al., 2007]. Cobalt at low levels exhibits little toxic potential, but may cause health concerns at high levels. Health effects related to high cobalt levels include cardiomyopathy [Van Vleet & Ferrans, 1986] and decreased weight gain [Huck & Clawson, 1976]. Depending on the route of exposure, nickel can impose systemic, immunologic, neurologic, reproductive, developmental, or carcinogenic effects [Das et al., 2008; Eisler, 1998; Outridge & Scheuhammer, 1993]. No clinical signs were noted on physical exams or laboratory analysis to indicate such health issues; however, the effects of chronic low-level exposure of metals on lemurs are as yet unknown.

Indri at BSNR and AFC differed in several physiological parameters, some of which could indicate stimulated immune response and reduced nutritional quality. Indri at AFC exhibited significantly higher total WBC count, segmented neutrophil count, and lymphocyte count compared with BSNR. Elevation of WBC, segmented neutrophils, or lymphocytes indicates an immune system response to infection or inflammation. In contrast, globulin levels were lower for AFC indri compared with BSNR. In the face of immune system, stimulation globulin levels would be expected to increase. The explanation of these contradicting indicators of immune function is not clear. Factors influencing the elevated immune response may include the higher parasite diversity found within AFC indri or the reduced habitat quality at AFC, or a combination of both. If parasitemia were a primary cause of this increase, eosinophilia would be expected; however, this was not present. AFC indri undergo more exposure to humans, and therefore more opportunity for pathogen pollution. Degraded habitat has been shown to compromise health and increase susceptibility to infection [Chapman et al., 2006b]. Although we might expect to see a difference in body mass owing to compromised health or reduced nutritional availability at the more degraded AFC, we saw no significant differences in mean weight.

Significant serum chemistry differences were noted in ALT and SAP, which were both higher in AFC indri relative to BSNR. These enzymes may be associated with liver disease or injury. Both sets of values are within the range generally accepted for mammals [Tennant, 1997], suggesting these differences are within normal variation and not an indication of compromised health. AFC individuals were also significantly lower than BSNR for the following serum chemistries: protein values (total protein, albumin, and globulin), calcium, chloride, creatinine phosphokinase (CK), and creatinine. Lower protein values and electrolytes noted within the AFC population may reflect poor nutrition, suggesting dietary intake of these nutrients may be lower owing to the poorer quality secondary forest at AFC. Indri at AFC did maintain higher values for magnesium, which is also related to dietary intake. CK is a muscle enzyme released immediately in response to muscle damage, such as exertion. Values for CK at AFC were approximately one/third lower than those for BSNR, which may be explained by behavioral differences between indri at the two sites. Because indri at AFC are habituated to humans, they did not flee when the dart team approached, and also did not travel far after being darted. Indri at BSNR displayed a more typical flight response and may have exerted more, resulting in elevated CK levels.

Iron analytes, fat-soluble vitamin, and trace mineral differences further indicate variation in dietary intake between these two sites; however, detailed analysis of nutrient composition of dietary items is not available to confirm these differences. Variation in nutrient composition may be owing to soil composition, plant selection, seasonal shifts in plant availability, or reduced accessibility to quality feeding areas. Serum iron analyte values (iron, TIBC, ferritin, transferrin saturation) were not significantly different between sites. Serum iron analyte determination is a useful measure of iron metabolism, deficiency, or excess. Values for indri at both sites fall within general mammal ranges (serum iron 55–185 μg/dl, TIBC 250–425 μg/dl, T-sat 33%). Serum iron analytes are used to reflect body iron stores in humans and may be applied to some animal species [Lowenstine & Munson, 1999; Tennant, 1997]; however, recent data suggest that the value of these parameters varies markedly among lemur species [Williams et al., 2008].

Fat-soluble vitamins were not significantly different between sites, with the exception of vitamin A, which was higher at AFC. These values fell within normal ranges for other lemur species; however, there is a consistent lack of carotenoids in all samples, indicating that lemurs may metabolize or utilize these compounds differently than other species. This is consistent with vitamin analyses from other wild lemur species [Dutton et al., 2003, 2008; Junge & Louis, 2005b, 2007; Junge et al., 2008], but different from most primate species that have detectable carotenoids [Crissey et al., 1999; Slifka et al., 1999]. In fact, most primates are considered carotenoid accumulators, whereas ungulates are more commonly considered nonaccumulators [Slifka et al., 1999]. This supports the suggestion that vitamin metabolism in prosimians is clearly different than other primates.

No differences were 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. If indri relied on dermal conversion for a significant amount of vitamin D, differences between these populations might be expected owing to seasonal differences in sun exposure. These similar values may suggest

that indri are able to utilize dietary sources of vitamin D.

Zinc and manganese measured significantly higher for AFC individuals, but selenium values were 40% lower at AFC. Despite these differences, these values remain within normal ranges for mammals [Kaneko et al., 1997], including other lemur species [Dutton et al., 2003, 2008; Junge & Louis, 2005b, 2007; Junge et al., 2008].

Enteric bacteria, typically considered to be pathogenic in lemurs (*Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*) [Bresnahan et al., 1984; Coulanges, 1978; Lhuillier & Zeller, 1978; Luechtefled et al., 1981; Obwolo, 1976], were not detected. Detection of these pathogens in lemurs could indicate transmission from humans or their associated animals or indirect exposure via fecal contamination in the environment. Human- associated pathogens have been identified in wild primate populations, including *E. coli* in primates in Uganda [Goldberg et al., 2008b] and enteric pathogenic bacteria [Nizeyi et al., 2001] and sarcoptic mange in mountain gorillas (*Gorilla gorilla berengei*), and may possibly be related to ecotourism [Kalema-Zikusoka et al., 2002]. The recent emergence of a lemur bushmeat market in Madagascar not only enhances the risk of pathogen pollution owing to increasing human exposure, but also heightens the danger of zoonotic disease emergence within human populations owing to the close bodily contact associated with the bushmeat hunting process [Barrett & Ratsimbazafy, 2009; Golden, 2009; Wolfe et al., 2005].

Toxoplasmosis has been reported in lemur species and often results in high mortality rates that vary with lemur species [Dubey et al., 1985; Junge & Louis, 2007; Junge et al., 2008]. Serum samples from both BSNR and AFC were negative for *T. gondii*. The definitive hosts for *T. gondii* are felid species; therefore, detection of titers in lemurs would indicate exposure to domestic cats as there are no native felids in Madagascar. Alternatively, lack of seropositivity could indicate that indri die acutely of toxoplasmosis (as *L. catta* do), rather than survive and mount a serological titer (as seen with *Varecia*). Serosurvey for a variety of viral pathogens (herpesvirus SA8, simian retrovirus 2, simian T-lymphotropic virus, simian immunodeficiency virus, simian foamy virus, and measles) was negative. None of these viral diseases are known to exist in lemurs; however, close human contact via mining, logging, hunting, or tourism creates opportunities for transmission. Serological assays were selected owing to cross-reactivity (SA8 reacts with a variety of herpesviruses), presence in the human population (measles), or virus group represented (foamyvirus, retrovirus, immunodeficiency virus). The assay is a nonspecies-specific antigen blocking assay; therefore, cross-reaction with nonsimian primate sera is expected. However, because these viral diseases have never been documented nor experimentally produced in lemurs, no positive controls are available. Therefore, negative results may simply indicate that the assay does not work in this species.

Ectoparasites can compromise health in an infected individual and can act as vectors for harmful pathogens, such as arboviruses, *Bartonella*, plague, murine typhus, and *Ehrlichia* spp., all of which have been documented within Madagascar [Duplantier & Duchemin, 2003; Rousset & Andrianarivelo, 2003]. In this study, ectoparasites varied greatly between sites, with indri at AFC exhibiting higher prevalence for all ectoparasites except for the mite, *L. madagascariensis*. At BSNR, only *L. madagascariensis* was identified, whereas at AFC, three types of ectoparasites were identified, including mites, lice (*T. babakotophilus*), and ticks (*H. lemuris* and *Ixodes*). Only *T. babakotophilus* was considered to be significantly higher at AFC than at BSNR; however, this outcome may have simply been a result of limited sample sizes. At AFC, two individuals suffered from multiple infections of more than one ectoparasite type, and 37.5% individuals harbored ectoparasites compared with only 10% at BSNR. The heavy ectoparasite load at AFC may indicate compromised health, increased exposure to ectoparasite transmission, or suboptimal habitat.

Samples were collected in May and October, which introduces seasonal and climatic variation. Seasonality will play a role in nutritional availability, lemur activity levels, exposure to parasites within the environment as well as parasite life cycles [Altizer et al., 2006; Nunn & Altizer, 2006]. Although available forage varies between these sites, no significant differences were detected in body weight between populations. In BSNR, the rainy season ends just before May, and in ARC, October falls within the middle of the dry season. One would predict elevated rates of parasitism during the wet season, as increased precipitation and temperature influence parasite transmission and survival in the environment [Altizer et al., 2006]. However, indri at BSNR actually had lower parasite prevalence and richness.

This study suggests that indri under greater anthropogenic disturbance exhibited physiological changes compared with indri in a pristine forest. Long-term, consistent monitoring of the effects of disturbance will be critical to ensuring the survival of these populations. The need for this monitoring is only heightened by the endangered status of Madagascar's lemurs, as well as the projected increase in anthropogenic pressure in the future. With this type of monitoring, it may be possible to recognize the cumulative and interactive effects of a combination of stressors on wildlife populations, including environmental contamination, reduced nutritional quality, pathogen pollution from humans and domestic animals,

susceptibility to disease, hunting, and habitat loss and degradation. Possessing knowledge about how habitat degradation and contamination from mining affects lemurs and other wildlife will assist protected area managers in addressing the health and sustainability of wildlife populations.

ACKNOWLEDGMENTS

We thank Madagascar National Parks (MNP) and the Mitsinjo Association for permission to conduct this research, the Madagascar Institute for the Conservation of Tropical Ecosystems (MICET) and Madagascar Fauna Group (MFG) for logistical assistance in Madagascar, and the Madagascar Biodiversity Project Field Team for field support. We also thank A. Junge, A. Greven, T. Rakotonanahary, and H. Rafalinirina for assistance in the field and D. Valle and the Yoder Lab for helpful feedback. Site knowledge, as well as logistical and field support, was additionally provided by the staff of the Mitsinjo Association, Dr. Rainer Dolch, Christin Nasoavina, and Clementine. This project was funded in part by the St. Louis Zoo Field Conservation for Research Fund and the National Science Foundation. Activities in this project complied with protocols approved by the St. Louis Zoo and Duke University's Institutional Animal Care and Use Committee, as well as adhered to all research requirements in Madagascar and CITES regulations.

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