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# GASTROINTESTINAL PARASITES OF CAPTIVE AND FREE-LIVING LEMURS AND DOMESTIC CARNIVORES IN EASTERN MADAGASCAR

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*Abstract:* Fecal samples from captive and free-living lemurs at Ivoloina Zoological Park (IZP) and domestic carnivores from six villages surrounding IZP were evaluated between July and August 2012. Free-living lemurs from Betampona Natural Reserve (BNR), a relatively pristine rainforest fragment 40 km away, were also evaluated in November 2013. All 33 dogs sampled (100%) and 16 of 22 cats sampled (72.7%) were parasitized, predominantly with nematodes (strongyles, ascarids, and spirurids) as well as cestodes and protozoans. Similar types of parasites were identified in the lemur populations. Identification of spirurid nematodes and protozoans in the lemur fecal samples were of concern due to previously documented morbidity and mortality in lemurs from these parasitic agents. Twelve of 13 free-living (93%) and 31 of 49 captive (63%) lemurs sampled at IZP had a higher parasite prevalence than lemurs at BNR, with 13 of 24 (54%) being parasitized. The lemurs in BNR are likely at risk of increased exposure to these parasites and, therefore, increased morbidity and mortality, as humans and their domestic animals are encroaching on this natural area.

*Key words:* *Eulemur fulvus albifrons*, human–domestic animal–wildlife interface, *Indri indri*, *Propithecus diadema*, *Spirocerca lupi*, *Varecia variegata*.

## INTRODUCTION

Ancestral lemurs were the first terrestrial mammals to colonize Madagascar and over a period of at least 50 million years evolved to fill the island's different ecological niches.[8,33,46] Today, the majority of lemur species are threatened with extinction from human-mediated environmental change.[1,20] While deforestation, habitat fragmentation, and bushmeat hunting are leading causes of population declines, small populations are vulnerable to extirpation from stochastic events, including diseases that reduce fecundity, fitness, and survival.[6,18,21,23]

Studies evaluating the health of free-living lemurs have been conducted for decades with the objectives to establish baseline health parameters and to provide continual health monitoring.[11,12,24–27] Despite the extensive research, few infectious diseases have been identified. However, parasites, including gastrointestinal, ectoparasites, and hemoparasites, have been detected, and a number of lemur species have tested serologically positive for West Nile virus.[11,17,22,26,28,36,40] Increasingly, studies have focused on the effects of environmental anthropogenic change on lemur health, demonstrating that lemurs in relatively pristine forests are more fit and have lower parasite loads than those in smaller fragmented forests with increased exposure to humans and their associated animals.[21,23,34]

In captivity, lemurs have been diagnosed with a number of parasitic diseases that include but are not limited to fatal neurologic toxoplasmosis; *Baylisasaris procyonis* neural larval migrans; *Physaloptera* spp. stomach worm infestation; *Echinococcus granulosus* and *E. multilocularis* hydatid disease; *Taenia crassiceps* cysticercosis; aortic aneurysm and rupture due to *Spirocerca lupi* larval migration; and diarrhea associated with *Cryptosporidium* spp. and *Giardia* spp.[2,5,9,13,30,39,41,43,44]

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During the past few decades, many cross-taxa studies have been conducted to evaluate the prevalence of diseases at the wildlife, human, and domestic animal interface.[6,7,38,42] For example, in Bolivia, domestic dogs (*Canis lupus familiaris*) and cats (*Felis catus*) and a number of nondomestic carnivore species living near and within protected parks were antibody positive for canine distemper virus, canine parvovirus, feline calicivirus, and *Leptospira interrogans*.[3,14,15] The authors concluded in each of these studies that disease transmission was occurring between domestic and nondomestic carnivores.[3,14,15] Recent studies in free-living lemurs in Madagascar demonstrated positive titers to *Toxoplasma gondii* in white-fronted brown lemurs (*Eulemur fulvus albifrons*) in Nosy Mangabe Special Reserve, *Cryptosporidium* spp. in greater bamboo lemurs (*Prolemur simus*), and brown mouse lemurs (*Microcebus rufus*) in Ranomafana National Park.[24,35] Pathogenic enterobacteria and viruses were also found in lemurs in Ranomafana National Park.[4,48]

Although parasites of domestic carnivores are known to cause disease in captive lemurs, minimal data are currently available on gastrointestinal parasites of domestic carnivores in Madagascar.[9,13,30,39,41,43,44] These data will be valuable as a baseline reference, especially as domestic animals are increasingly encroaching into Madagascar forests (Rasambainarivo, pers. comm.). The primary objective of this study was to determine the presence and prevalence of gastrointestinal parasites of both captive and free-living lemurs as well as sympatric domestic carnivores at a zoological park and free-living lemurs in a protected reserve in Madagascar. Additionally, a questionnaire survey was utilized to understand the demography of the domestic animals. To the authors' knowledge, this is the first study to evaluate gastrointestinal parasites of captive and free-living lemurs and domestic carnivores across a gradient of interspecies contact in Madagascar.

## MATERIALS AND METHODS

### Study sites

Fecal samples were collected from two primary sites in Madagascar: Ivoloina Zoological Park (IZP) along with six of its surrounding villages and Betampona Natural Reserve (BNR) (Fig. 1). IZP is located on the east coast, 14 km north of the city of Toamasina (S18.1500, E49.4167). The park is 282 ha, and the number of lemurs in the collection fluctuates but at the time of the study consisted of 75 individuals from 12 species. The cages contained natural sand substrate that was cleaned daily, and the animals were fed fresh browse and produce. The captive lemurs received anthelmintic therapy consisting of ivermectin (0.2 mg/kg p.o. once a month for 3 mo) or fenbendazole (50 mg/kg p.o. s.i.d. for 3 days repeated monthly for 3 mo) on a rotational schedule (3-mo interval between treatments) as part of the preventive health program. In addition, there were free-living lemurs at IZP that did not receive anthelmintics.

Local people were interviewed and fecal samples were collected from the domestic carnivores in the villages of Ambonivato, Bemenaka, Anjaridaina, College, Antsampanana, and Carriere, which were all within 1 km of IZP (Fig. 2).

BNR (S17.931389, E49.20333) is a low-altitude, mountainous, evergreen rain forest approximately 40 km northwest of Toamasina. This 2,228-ha relatively pristine forest is home to 11 species of lemurs; five endemic species of carnivores, including the largest endemic carnivore to Madagascar, the fosa (*Cryptoprocta ferox*); and a large variety of birds, reptiles, amphibians, and invertebrates (Madagascar Fauna and Flora Group inventory, unpubl. data).

### Sample collection and evaluation

In July and August 2012, household surveys ($n = 84$) of the villages surrounding IZP were performed by interviewing household members (Table 3). Information regarding ownership, purpose (i.e., hunting, dog fighting), demographics, and basic health of domestic animals was obtained. Questions regarding observance of free-living lemurs and feral dogs within the villages were also asked. Fecal samples from individual dogs and cats were collected at the time of the interview.

During the same time period, fecal samples from lemurs at IZP were collected for evaluation. Opportunistic fecal samples were collected from 49 captive lemurs, consisting of *Cheirogaleus* sp. (dwarf lemurs) ($n = 3$), *Daubentonia madagascariensis* (aye-aye) ($n = 1$), *Eulemur fulvus rufus* (red fronted lemur) ($n = 2$), *E. f. fulvus* (common brown lemur) ($n = 7$), *E. coronatus* (crowned lemur) ($n = 1$), *E. f. albifrons* (white-fronted brown lemur) ($n = 5$), *E. rubriventer* (red-bellied lemur) ($n = 3$), *E. macaco flavifrons* (blue-eyed black lemur) ($n = 10$), *Hapalemur griseus* (eastern lesser bamboo lemur) ($n = 8$), *P. simus* (greater bamboo lemur) ($n = 2$), *Microcebus* sp. (mouse lemurs) ($n = 1$), and *Varecia variegata* (black-and-white ruffed lemur)

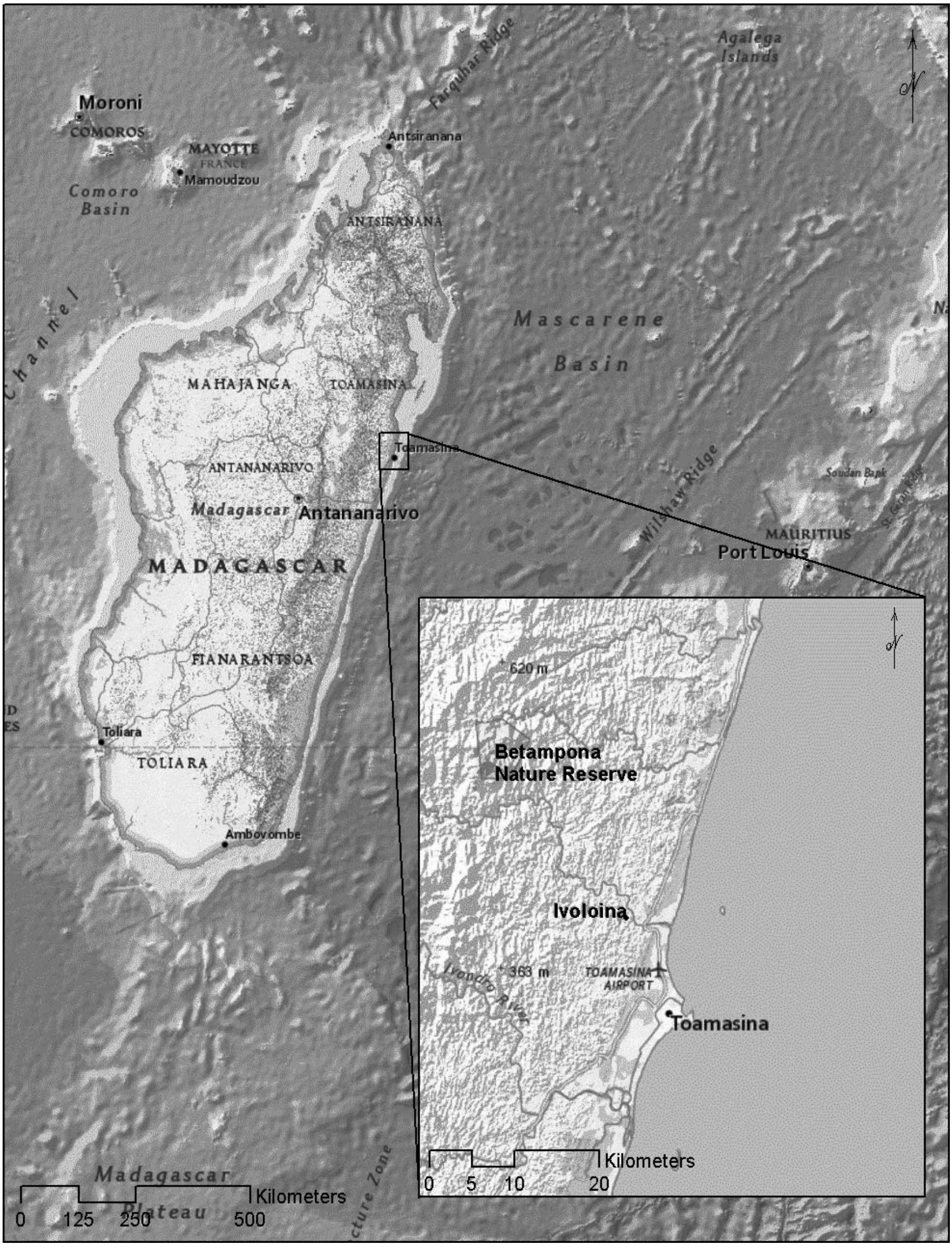


**Figure 1.** Map of Ivoloina Zoological Park and Betampona Natural Reserve in Madagascar.

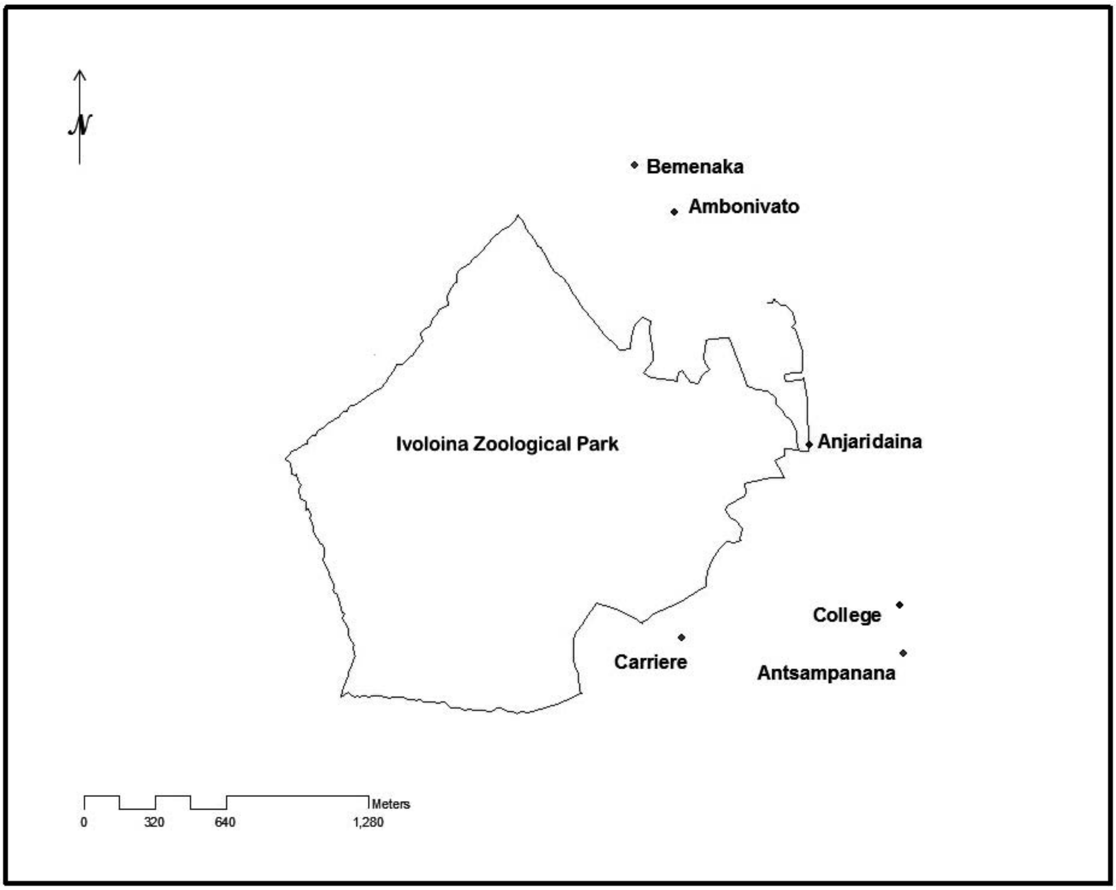


**Figure 2.** Map of the perimeter of Ivoloina Zoological Park and the surrounding villages surveyed in Madagascar.

($n$ = 6). Opportunistic fecal samples were also collected from 13 free-living lemurs on zoo grounds, consisting of *E. f. albifrons* ($n$ = 8), *E. rubriventer* ($n$ = 3), and *V. variegata* ($n$ = 2).

In October 2013, fecal samples were collected from four lemur species as part of a concurrent research study in BNR: *V. variegata* ($n$ = 2), *E. f. albifrons* ($n$ = 3), *Indri indri* (indri) ($n$ = 1), and *Propithecus diadema* (diademed sifaka) ($n$ = 1) in BNR. Freshly voided fecal samples were also collected opportunistically from free-living *V. variegata* (n = 2), *E. f. albifrons* ($n$ = 1), *I. indri* ($n$ = 9), and *P. diadema* ($n$ = 4) in BNR.

The fecal samples from domestic dogs and cats and lemurs at IZP and BNR were evaluated by light microscope within 24 hr of collection by a standard floatation technique.[16] Egg morphology was used for parasite identification based on parasites of domestic animals and previously identified parasites of lemurs.[22]

### Statistics

Fecal samples were collected from identifiable individual animals, and none of the individuals were sampled more than once; thus, all samples were independent. The prevalence of parasites was determined for each group. Fisher exact two-tailed tests were performed to determine if there was a statistically significant difference ($P < 0.05$) in parasite prevalence between the IZP captive and free-living lemurs and IZP and BNR free-living lemurs (NCSS, Kaysville, Utah 84037, USA).[47]

## RESULTS

### Household surveys and domestic carnivores

Of the 84 households surveyed, 52 owned dogs (24 males, 28 females) and 35 owned cats (13 males, 22 females). Fecal samples were collected from 33 dogs (14 males, 19 females) and 22 cats (6 males, 16 females). Thirty-six of the households

**Table 1.** Questionnaire derived demographic data (mean ± SD) and use (95% confidence interval) for domestic dogs and cats in six villages surrounding Ivoloina Zoological Park.

| Questionnaire parameter | Responses | |
|---|---|---|
| | Dogs ($n = 52$) | Cats ($n = 35$) |
| No. of animals per household | 0.65 ± 1.1 | 0.43 ± 0.7 |
| Age (mo) | 21.2 ± 23.9 | 23.3 ± 20.8 |
| Sex ratio (male to female) | 0.85 : 1 | 0.59 : 1 |
| Age of death (mo) | 31.1 ± 25.9 | 28.5 ± 31 |
| Litter size | 3.6 ± 1.6 | 2.7 ± 0.9 |
| No. of young that die per litter | 1.3 ± 1.3 | 1.6 ± 1.4 |
| Animals vaccinated | 0 | 1 (0.07–14.9) |
| Animals surgically sterilized | 0 | 1 (0.07–14.9) |
| Animals received anthelmintic | 1 (0.05–10.3) | 6 (6.6–33.6) |
| Animals used for hunting | 30 (43.2–71.3) | 35 (90–100) |
| Animals used for fighting | 14 (15.6–41) | — |
| Animals that roam | 37 (57–82.9) | — |

(42.9%) reported feral dogs, and 21 (25%) reported free-living lemur sightings within their villages. The demographic data, basic medical history, and purpose of the domestic carnivores are reported (Table 1).

All of the 33 dogs sampled (100%) had parasites with a minimum of two types recognized in each sample. Parasites identified in order of highest prevalence were strongyles (31/33), ascarids (23/33), cestodes (18/33), trichurids (17/33), spirurids (12/33), coccidia (5/33), and protozoans (4/33) (Table 2).

Sixteen of the 22 cats sampled (72.7%) had parasites with 14 (63.6%) samples having more than one type recognized. Parasites identified in order of highest prevalence were strongyles (13/22), ascarids (7/22), cestodes (7/22), protozoans (3/22), trichurids (2/22), and coccidia (2/22) (Table 2).

### IZP captive and free-living lemurs and BNR free-living lemurs

Of the 49 fecal samples from captive IZP lemurs, 31 (63.3%) had parasites. Parasites identified in order of highest prevalence were oxyurids (25/49), strongyles (16/49), spirurids (3/49), trichurids (1/49), and coccidia (1/49). Of the 13 fecal samples from the free-living lemurs at IZP, 12 (92.3%) had parasites. Free-living lemurs at IZP had parasites at a statistically significant higher prevalence than the captive IZP lemurs ($P = 0.049$). Parasites identified in order of highest prevalence were oxyurids (11/13), strongyles (7/13), coccidia (2/13), and spirurids (1/13) (Table 2).

Thirteen of the 24 sampled free-living lemurs (54.2%) in BNR had parasites. Overall, parasites identified in order of highest prevalence were strongyles (10/24), ciliated protozoans (3/24), and oxyurids (1/24) (Table 2).

IZP's free-living lemurs also had a statistically significant higher prevalence of parasitism than the BNR free-living lemurs ($P = 0.027$).

## DISCUSSION

As humans and domestic animals encroach on naïve ecosystems, sympatric wildlife species are at risk of exposure to the parasitic and infectious agents harbored by people and their animals alike. In this study, the lemurs (both captive and free-living) in closer proximity to humans and their domestic animals had a higher prevalence and variety of parasitism than those living in the more protected forest.

The household surveys showed that dogs and cats suffered a high neonatal mortality rate, and of those that survived, the average life span was short at approximately 2.5 yr of age. This may result in continual recruitment of young, immunologically naive animals for parasites and pathogens and potential for transmission. Domestic carnivores were most commonly used for bushmeat hunting, received minimal veterinary care, and roamed far from home, all of which may support transmission of parasites and other infectious agents to sympatric wildlife species. This is especially concerning since all of the dogs and approximately 75% of the cats sampled had gastrointestinal parasites. The most prevalent types of parasites identified were nematodes (strongyles, ascarids, trichurids, and spirurids) as well as cestodes and protozoans. Although there have been reports of *T. gondii* in domestic cats, humans, and endemic carnivores in Madagascar,

**Table 2.** Parasite prevalence (95% confidence interval) of domestic dogs and cats and captive and free-living lemurs in Madagascar.

| Group | *n* | Parasitized | Strongyles | Ascarids | Trichurids | Spirurids | Oxyurids | Cestodes | Coccidia | Protozoans |
|---|---|---|---|---|---|---|---|---|---|---|
| Dogs | 33 | 33 (89.4–100) | 31 (70.8–99.3) | 23 (51.3–84.4) | 17 (33.5–69.2) | 12 (20.4–54.9) | — | 18 (36.4–71.9) | 5 (5.1–31.9) | 4 (3.4–28.2) |
| Cats | 22 | 16 (49.7–89.3) | 13 (36.4–79.3) | 7 (13.9–54.9) | 2 (1.1–29.2) | — | — | 18 (13.9–54.9) | 2 (1.1–29.2) | 3 (2.9–34.9) |
| Ivoloina captive lemurs | 49 | 31 (48.3–76.6) | 16 (19.9–47.5) | — | 1 (0.05–10.9) | 3 (1.3–16.9) | 25 (36.3–65.6) | — | 1 (0.5–10.9) | — |
| Ivoloina free-living lemurs | 13 | 12 (64–99.8) | 7 (23.1–80.8) | — | — | 1 (0.2–36.0) | 11 (54.6–98.1) | — | 2 (1.9–45.4) | — |
| Betampona free-living lemurs | 24 | 13 (32.8–74.4) | 10 (22.1–63.4) | — | — | — | 1 (0.11–21.1) | — | — | 3 (2.7–32.4) |

the protozoan eggs identified in this study were not morphologically consistent with *T. gondii*.[29,32]

Both the captive and free-living lemurs at IZP were parasitized, mostly with strongyles and oxyurids, similar to a previous report.[36] The free-living lemurs at IZP had higher parasite prevalence and intensity compared to the captive IZP lemurs, which fits with the lack of anthelmintics for this population and the probable closer contact they have with local humans and domestic carnivores (Deem and Rasambainarivo, unpubl. data). A small percentage of both groups had spirurid eggs in their feces, which were morphologically consistent with *S. lupi*. This parasite has been reported to cause aortic aneurysms in lemurs similar to that observed in domestic dogs.[2] Adult worms usually reside in the walls of the esophagus, stomach, or aorta, but the larvae can then migrate through the celiac artery into the thoracic aorta. Granulomas of developing larvae within the aorta may result in aneurysm and aortic rupture.[31] This finding is of particular concern, as there have been recent cases of sudden death with thoracic hemorrhage in both captive and free-living *V. variegata* and *Eulemur* spp. at IZP, presumptively caused by *S. lupi* (Deem and Rasambainarivo, unpubl. data).

A low prevalence of trichurids was found in the captive lemurs. Trichurids were also found in the domestic dogs, most likely consistent with *Trichuris vulpis*, the canine whipworm. Although *Trichuris* spp. are generally considered host specific, there are reports of *T. vulpis* infection in humans (i.e., cross-species transmission), which may result in diarrhea and dehydration.[10] Therefore, the lemurs in this study may also be at risk from this potential zoonotic parasite.

All the BNR free-living lemur species tested had gastrointestinal parasites. The three groups of parasites seen in the BNR lemurs have been previously documented in free-living lemurs, but pathogenicity is unknown.[23,26] It is possible that they are nonpathologic commensals with a symbiotic relationship. However, if a lemur host became immunocompromised from a stressor (i.e., a coinfection, habitat fragmentation, domestic animal encroachment), a commensal organism may increase in both intensity and pathogenicity.[37] We also found that lemurs in the center of BNR are not isolated from domestic animals, as during our fieldwork, domestic dogs were heard barking within the reserve and recent camera trap data confirm the presence of domestic carnivores in BNR (Rasambainarivo, unpubl. data).

**Table 3.** Questionnaire administered to gather demographic data, basic health, and purpose of domestic dogs and cats in villages surrounding Ivoloina Zoological Park.

| Question |
|---|
| How many domestic dogs and/or cats do you own? |
| What is the age of each animal? |
| What is the gender of each animal? |
| What was the age of any of your dogs and/or cats that died in the past 12 mo? |
| How many puppies/kittens were in the most recent litter? |
| How many of the puppies/kittens died within the first 2 mo of life? |
| How many of the dogs and/or cats have been vaccinated? |
| How many of the dogs and/or cats have been surgically sterilized? |
| How many of the dogs and/or cats have received an anthelmintic? |
| How many of the dogs and/or cats are used for hunting? |
| How many of the dogs and/or cats are used for fighting? |
| How many of the dogs and/or cats roam far from home? |
| Have you seen feral dogs in your village? |
| Have you seen free-living lemurs in your village? |

We identified parasites in the same genus for both the domestic carnivores and all lemur groups evaluated, and it is possible that transmission of these parasites may be occurring across taxa.[30,39,43,44] However, other potential sources of parasite exposure to the lemurs should be considered. Human pathogens have been shown to infect free-living nonhuman primates. Due to the closer phylogenetic relationship of humans and lemurs as compared to domestic carnivores and lemurs, humans may be more likely than domestic animals to share parasites with prosimians.[45] Intrinsic or ecological stressors may also cause immunocompromise of captive and free-living lemurs at IZP, increasing susceptibility to parasites and/or intensity of endemic parasites. These may include dietary differences from the free-living lemurs in BNR, unidentified infectious processes, and population genetic differences. Poor hygiene from humans handling the food sources, improper cleaning of food bowls, fecal contamination of water from humans or livestock runoff, and rodents or invertebrates entering the enclosures are also potential sources for parasite transmission.

Limitations to this study include nematode parasite identification to the genus level and not to species since morphologic egg characteristics preclude this level of classification. Additionally, cestodes and protozoans could not be further classified. Finally, the study would be strengthened by larger sample sizes, particularly for free-living lemurs at BNR.

These data are presented as a baseline reference. They may also serve as a template for further studies of other lemur populations, humans, and/or domestic animal health in Madagascar so that we may better understand the potential causes that lead to an increased parasite prevalence in the lemurs living in close proximity to humans and domestic animals. The role of domestic carnivores warrants special attention, as it has been shown that lemurs are susceptible to parasites of domestic carnivores, encroachment of domestic carnivores throughout lemur habitat is increasing, and little data exist on parasites of domestic carnivores in Madagascar. To confirm cross-species transmission, we recommend that future studies include species-level parasite identification using molecular markers. Additionally, identification of human gastrointestinal parasites; identification of gastrointestinal parasites of ungulates and rodents; *Giardia* and *Cryptosporidium* testing of humans, domestic animals, and lemurs; and comparison of stress markers between captive and free-living lemurs by evaluating blood work and fecal cortisol levels should be conducted.

In conclusion, although similar gastrointestinal parasites were found in the domestic carnivores and lemurs, free-living lemurs in closer vicinity to humans and their domestic carnivores had a higher prevalence and variety identified than those in the more pristine area of BNR. This supports the concern that as humans and their domestic animals encroach on the remaining forests of Madagascar, the lemurs in these areas may be increasingly exposed to parasitic and infectious agents they carry. Slowing deforestation, educating people, controlling domestic animals and humans within reserves, and monitoring the health of lemur populations are essential for the long-term survival of these iconic animals.

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