

# HEALTH ASSESSMENT OF WILD LOWLAND TAPIR (*TAPIRUS TERRESTRIS*) POPULATIONS IN THE ATLANTIC FOREST AND PANTANAL BIOMES, BRAZIL (1996–2012)

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**ABSTRACT:** The lowland tapir (*Tapirus terrestris*) is found in South America and is listed as Vulnerable to Extinction by the International Union for Conservation of Nature, Red List of Threatened Species. Health issues, particularly infectious diseases, are potential threats for the species. Health information from 65 wild tapirs from two Brazilian biomes, Atlantic Forest (AF) and Pantanal (PA), were collected during a long-term study (1996–2012). The study included physic, hematologic and biochemical evaluations, microbiologic cultures, urinalysis, and serologic analyses for antibodies against 13 infectious agents (viral and bacterial). The AF and PA tapirs were significantly different for several hematologic and biochemical parameters. Ten bacteria taxa were identified in the AF and 26 in the PA. Antibodies against five viruses were detected: Bluetongue virus, eastern equine encephalitis virus, western equine encephalitis virus, infectious bovine rhinotracheitis virus, and porcine parvovirus. A high prevalence of exposure to *Leptospira interrogans* (10 serovars: Autumnalis, Bratislava, Canicola, Copenhageni, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, and Pyrogenes) was detected in both the AF and PA sites. A greater diversity of serovars and higher antibody titers were found in the PA. Statistically significant differences between sites were found for *L. interrogans*, equine encephalitis virus, and porcine parvovirus. Based on physical evaluations, both AF and PA populations were healthy. The differences in the overall health profile of the AF and PA tapir populations appear to be associated with environmental factors and infectious diseases ecology. The extensive datasets on hematology, biochemistry, urinalysis, and microbiology results from this paper can be used as reference values for wild tapirs.

**Key words:** Biochemistry, health, hematology, microbiology, serology, urinalysis, tapir, *Tapirus terrestris*.

## INTRODUCTION

Tapirs are a monophyletic assemblage of mammals that, along with horses and rhinoceroses, comprise the order Perissodactyla. There are four distinct tapir species that belong to the single genus *Tapirus*: two in South America (lowland tapir, *Tapirus terrestris*; and mountain tapir, *Tapirus pinchaque*), one in central and northern South America (Baird's tapir, *Tapirus bairdii*), and one in Southeast Asia (Malayan tapir, *Tapirus indicus*).

The lowland tapir occurs in 11 South American countries, 21 biomes, and numerous habitat types (Medici 2011). The species is listed by the International Union

for Conservation of Nature (IUCN) and the Brazilian Red Lists as Vulnerable to Extinction (Medici et al. 2012; IUCN 2013). Due to their individualistic lifestyle, low reproductive rate, long generation time, and low population density, tapirs are rarely abundant, which makes them more susceptible to threats such as habitat destruction and fragmentation, hunting, and road kill (Medici et al. 2007a; Medici and Desbiez 2012). Health issues, particularly infectious diseases, are also seen as potential threats to tapirs in the wild (Hernandez-Divers et al. 2005; Medici et al. 2007a). The proximity of lowland tapirs to domestic livestock in several parts of the species distribution creates opportunities

for disease transmission (Medici et al. 2007a).

Most of the information on tapir health comes from captive animals (Nunes et al. 2001; Mangini et al. 2002; Janssen 2003; Mangini 2007). The physiologic reference values used for tapirs have been compiled from captive tapirs (Teare 2006). Available information from wild tapirs comes from a long-term Baird's tapir study in Costa Rica (Hernandez-Divers et al. 2005) and from preliminary lowland tapir studies in Brazil (Furtado et al. 2010; Medici 2010; May 2011).

The lack of information about tapir health in the wild may be a major obstacle to planning effective strategies to improve tapir population survival and viability in the long term (Mangini et al. 2012; Medici and Desbiez 2012). Information about mortality and morbidity is essential to protect species from present and future threats (Deem et al. 2011).

The Lowland Tapir Conservation Initiative (LTCI), a pioneering, long-term tapir research and conservation program carried out by the Institute for Ecological Research (IPÊ) in Brazil, was first established in 1996 in the Atlantic Forest of São Paulo and expanded to the Pantanal in 2008. The research component of the LTCI includes studies on basic tapir biology, ecology, and population demography, as well as health, genetics, and threat assessments. We present tapir health information acquired by the LTCI from tapir populations in the Atlantic Forest (southern Brazil, 1996–2008) and Pantanal (central-western Brazil, 2008–12) and compare findings between study sites.

## MATERIALS AND METHODS

### Study sites

**Morro do Diabo State Park (MDSP)—Atlantic Forest:** The Atlantic Forest is one of the most threatened biomes on earth (Ribeiro et al. 2009). MDSP, an AF study site, is located in western São Paulo State (22°16'S, 52°05'W) and protects 370 km<sup>2</sup> of forest. The average

annual temperature is 22 C; annual rainfall is 1,347 mm (Faria and Pires 2006). MDSP is surrounded by a matrix of cattle ranches and agriculture, mostly sugar cane (Uezu et al. 2008). Smaller forest fragments (0.02–20 km<sup>2</sup>) are scattered around MDSP, creating a total of 127 km<sup>2</sup> of forest (Ditt 2002).

**Private Ranch—Pantanal:** The Pantanal is the largest continuous freshwater wetland on earth (160,000 km<sup>2</sup>). The private ranch, a PA study site, is located in the Nhecolândia subregion of the Pantanal, Mato Grosso do Sul (19°20'S, 55°43'W). The Pantanal is a mosaic of seasonally inundated grasslands, rivers, lakes, gallery forests, scrub, and deciduous forests that supports an abundance of wildlife. The average annual temperature is 25 C; annual rainfall is 1,185 mm (Calheiros and Da Fonseca 1996). Cattle ranching started in the mid-18th century and is the main economic activity in the region. Most of the livestock comprises Nelore cattle (*Bos indicus*) and horses (*Equus ferus caballus*).

### Capture and chemical restraint

Tapirs in both study sites were captured and anesthetized for fitting with radio collars. Additional procedures carried out during immobilization included subcutaneous insertion of microchips, morphometric measurements, sex and age determination, physical examination, and collection of biological samples. Three capture methods were used: 1) pitfalls, 2) box traps, and 3) darting from a distance using anesthetic darts (Medici 2010). Several anesthetic protocols were developed and tested (Mangini et al. 2001; Medici et al. 2007b; Table 1).

### Physical examination and sampling

Tapirs captured in box traps and pitfalls were visually inspected before anesthesia to estimate body mass and observe general condition, presence of lesions and scars, posture and ability to move, behavior, and signs of stress. All health parameters that could be obtained via visual inspection were recorded. Recaptured tapirs were visually evaluated and released immediately unless there was a need to anesthetize them (e.g., replacing or removing radio collar, collection of additional biologic samples).

Each anesthetized tapir received a complete physical examination that included 1) overall body condition (good, regular, or poor); 2) fur condition; 3) skin integrity (presence of scars or wounds, alterations in pigmentation); 4) examination of anatomic cavities (ophthalmic,

TABLE 1. Anesthetic protocols used for the chemical immobilization of wild lowland tapirs (*Tapirus terrestris*) in the Atlantic Forest (AF; 1996–2008) and Pantanal (PA; 2008–12), Brazil.

Anesthetic protocols (estimated doses, mg/kg) <sup>a</sup>	Capture method	n	Site
Detomidine (0.11)	Pitfall	1	AF
Medetomidine (0.008–0.01) + T/Z (4.11–5.6)	Pitfall	9	AF
Romifidine (0.09–0.11mg/kg) + T/Z (4.11–5.6)	Pitfall	3	AF
Detomidine (0.07–0.09) + T/Z (4.11–5.6)	Pitfall	2	AF
Detomidine (0.04–0.06) + ketamine (0.4–0.6) + T/Z (0.83–1.25)	Darting	4	AF
Medetomidine (0.004–0.006) + ketamine (0.4–0.6) + T/Z (0.83–1.25)	Darting	2	AF
Romifidine (0.03–0.05) + ketamine (0.4–0.6) + T/Z (0.83–1.25)	Darting	1	AF
Butorphanol (0.15) + medetomidine (0.003)	Box trap	12	AF
Butorphanol (0.15) + medetomidine (0.003) + ketamine (0.4–0.6) <sup>b</sup>	Box trap	5	AF
Butorphanol (0.15) + medetomidine (0.003) + azaperone (0.2) <sup>b</sup>	Box trap	2	AF
Butorphanol (0.04) + azaperone (0.8)	Box trap	3	AF
Medetomidine (0.004–0.006) + ketamine (0.4–0.6) + T/Z (0.83–1.25 mg/kg)	Darting	12	PA
Butorphanol (0.15) + medetomidine (0.003)	Box trap	23	PA
Butorphanol (0.15) + medetomidine (0.012) + ketamine (0.6)	Box trap	14	PA

<sup>a</sup> Atropine was added as needed (0.025–0.04 mg/kg); T/Z = Tiletamine/Zolazepam.

<sup>b</sup> Used as supplementary drug to induce recumbence or standing sedation.

nasal, ear, oral including dental evaluation, rectal); 5) palpation and auscultation; 6) evaluation of musculoskeletal integrity and mobility; 7) condition of nails and foot pads; 8) reproductive examination (in females, vaginal inspection, evaluation of mammary glands, evidence of reproductive activity; in males, evaluation of penis and palpation of testes); and 9) presence and level of infestation of ectoparasites. Individuals were assigned to age class based on dentition, tooth wear, erosion of nails, and appearance of foot pads. Age classes were: juvenile (6 mo–1 yr), sub-adult (>1–4 yr), and adult (>4 yr; Medici 2010).

Physiologic parameters including respiratory rate, cardiac rate, blood oxygen saturation, body temperature, level of muscle relaxation, response to stimuli, and palpebral and pupillary reflexes were monitored and recorded at intervals throughout anesthesia. Respiratory rate was monitored through auscultation of lungs. Cardiac rate and hemoglobin oxygen saturation were monitored through pulse oximetry (Nellcor 20A™, Covidien, Mansfield, Massachusetts, USA, or Oxy9Vet-Plus®, Bionet, Seoul, South Korea). Rectal temperature was obtained using a digital thermometer (Table 2).

Biological samples included blood, swabs of anatomic cavities (nasal, oral, ear, rectal, vaginal, urethra, and preputial) and active wounds. Blood samples were collected within 20 min of immobilization and consisted of an average of 50 mL collected through venipuncture on rami of saphenous, cephalic, or jugular veins using Vacutainer® tubes (nonadditive

and with ethylenediaminetetraacetic acid). In cases of spontaneous urination during anesthesia urine samples were collected in sterile tubes. Swabs were placed in Stuart transport medium. All samples were placed in a portable cooler on ice and immediately transported from the capture site to a field laboratory where they were preprocessed and properly stored for later laboratory analyses. Swabs were refrigerated and transported to a laboratory in <72 hr after collection. Blood samples were centrifuged and serum aliquots frozen at –18 C for up to 15 days before the end of the expedition and transportation to a laboratory. Urine samples were either analyzed in the field using urine test dip strips or transported to a laboratory.

**Laboratory analyses**

Hematologic, biochemical, microbiologic, and urine analyses were carried out in human

TABLE 2. Physiologic parameters of wild lowland tapirs (*Tapirus terrestris*) under anesthesia in the Atlantic Forest (AF; 1996–2008) and Pantanal (PA; 2008–12), Brazil.

Parameter	AF + PA		
	Mean	SD	n
Cardiac rate (beats/min)	75	18	60
Respiratory rate (breaths/min)	26	10	59
Blood oxygen saturation (%)	88	13	50
Body temperature (°C)	37	1	32

TABLE 3. Reference values for urinary parameters of wild lowland tapirs (*Tapirus terrestris*) in the Atlantic Forest (AF; 1996–2008) and Pantanal (PA; 2008–12), Brazil.

Parameter	AF			PA			AF + PA		
	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>
Specific gravity	1.013	0.0053	8	1.022	0.0052	6	1.017	0.007	14
pH	7.9	0.5	8	6.4	1.5	6	7.3	1.3	14
Epithelial cells/mL	1,142.9	378.0	7	1,000.0	612.4	5	1,083.3	468.7	12

diagnostic laboratories. In most cases the hematologic evaluation was performed manually. Parameters of the hematologic evaluation are listed in Supplementary Material Table S1. On some occasions, due to logistic reasons, part of the hematologic analyses was carried out in the field laboratory. Blood smears were prepared and stained with a Diff Quick stain kit (Laborclin, Pinhais, Brazil) for differential counts. Biochemical evaluation of serum was carried out using automated chemistry analyzers and included parameters listed in Supplementary Material Table S2.

Swabs of anatomic cavities and active wounds were cultured for aerobic and anaerobic bacteria. Urine analyses included param-

eters listed in Table 3 and Supplementary Material Table S3.

Serologic analyses were conducted for 13 viruses and bacteria relevant for tapir health and known in domestic livestock in both study sites. Table 4 lists diagnostic tests used for each infectious agent. Serologic tests were carried out in reference laboratories: 1998–2002: Centro de Diagnóstico Marcos Enrietti (Paraná); 2003–08: Laboratório Balagué (São Paulo); 2009–12: Instituto Biológico de São Paulo.

#### Data analysis

For the analyses of hematologic and biochemical parameters with sample sizes >40,

TABLE 4. Infectious agents, tests applied, laboratory used, and viral or bacterial strain evaluated in the serosurvey of wild lowland tapirs (*Tapirus terrestris*) in the Atlantic Forest (AF; 1996–2008) and Pantanal (PA; 2008–12), Brazil.

Infectious agent	Test <sup>a</sup>	Laboratory (strain) <sup>b</sup>
Bovine viral diarrhea virus	ELISA	IDEXX
Foot and mouth disease virus	AGID	(PANAFTOSA)
Equine infectious anemia virus	AGID	IDEXX
Bovine leukemia virus	AGID	EMBRAPA
Eastern equine encephalitis virus	Serum neutralization and virus neutralization VERO cells	(East)
Western equine encephalitis virus	Serum neutralization and virus neutralization VERO cells	(West)
Bluetongue virus	AGID	(PANAFTOSA)
Infectious bovine rhinotracheitis virus	Serum neutralization, MDBK (ATCC) AGID	(Los Angeles)
Pseudorabies virus (Suid herpesvirus type 1)	Serum neutralization in VERO cells	EMBRAPA (Concórdia)
Vesicular stomatitis virus	Serum neutralization and virus neutralization VERO cells	CDME (Indiana)
Porcine parvovirus	Hemagglutination inhibition	EMBRAPA (Concórdia)
<i>Leptospira interrogans</i>	Microscopic agglutination test	<i>L. interrogans</i> , 26 serovars
<i>Brucella</i> sp.	Plate serum agglutination, tube serum agglutination	TECPAR ( <i>Brucella abortus</i> )

<sup>a</sup> AGID = agar gel immune-diffusion; ELISA = enzyme-linked immunosorbent assay; MDBK = Madin and Darby bovine kidney cell; VERO = African green monkey kidney cell line.

<sup>b</sup> CDME = Centro Diagnóstico Marcos Enrietti; EMBRAPA = Empresa Brasileira de Agropecuária; IDEXX = IDEXX Laboratories; PANAFTOSA = Pan American Foot and Mouth Disease Center; TECPAR: Instituto Tecnológico do Paraná.

the median was used as a measure of central tendency, and the 2.5 and 97.5 percentiles were used to specify the 95% reference interval (CSLI 2008). The central 95% provided an estimate of the normal range (Rhoads 2007). For parameters with <40 samples, outlier cuts were applied and determined by the one-third rule, derived from a test originally proposed by Dixon (1953) and recommended by CLSI (2008). Recapture data were used only when recapture events took place at intervals longer than 30 days.

Hematologic and biochemical variables were tested for normality (Shapiro-Wilk test) and variance homogeneity (Levene's test), and compared by groups (study site > sex > age class) using parametric analysis of variance, when data were normal and homogeneous before or after transformation ( $\log_e$ ). The nonparametric Kruskal-Wallis test was used when data were not normal, even after transformation; alpha was set to 0.05. Statistical differences between the anesthetic protocols could not be evaluated due to the unbalanced samples. Hematologic and biochemical results were also tested against tapir reference values from ISIS (Teare 2006), "all ages all classes" category, through a single sample *t*-test.

Values for hematology and biochemical parameters were expressed in SI units and the following data were presented for each parameter evaluated: valid sample size (*N*), mean, range (minimum and maximum values), lower quartile (Q1), median, upper quartile (Q3), SD, and SE. Physical examination, physiologic, and urinary parameters were tabulated and presented descriptively.

The bacterial relative prevalence in anatomic cavities and lesions was analyzed for each study site and each cavity. The diversity of bacteria in each study site and the similarity between sites were measured by the Jaccard similarity coefficient (*S<sub>j</sub>*; Magurran 1988, 2004), where *S<sub>j</sub>*=1 would mean that both study sites had the same microbiologic profile, and *S<sub>j</sub>*=0 would mean that they had completely different microbiologic profiles (Wilson and Mohler 1983). Prevalence of bacterial strains present in both study sites was compared through a  $\chi^2$  (chi-square) test.

To evaluate the prevalence of serologic responses to infectious agents, the tapir population in the AF was estimated to be 130 individuals (Medici 2010). The study in the PA is ongoing, and population size estimates for the study site are preliminary (60–100 individuals; E.P.M. unpubl. data). The sampling prevalence was calculated as the proportion of tapirs with positive antibody responses in each study site (annually and for the entire study period). The

95% confidence intervals (CIs) were calculated as a "simple random sampling," according to sample size (small, medium, or large) and proportion of estimated population size (Thrusfield 2007). When prevalence was <5%, CI was calculated according to Wilson (1927), as recommended by Thrusfield (2007). When prevalence was 0 or 100%, the Clopper and Pearson (1934) methodology was applied. Chi-square tests were used to compare prevalence data by study site.

## RESULTS

Sixty-five individual lowland tapirs were evaluated: 35 in the AF (20 females, 15 males) and 30 in the PA (10 females, 20 males). Ninety-three chemical immobilizations were carried out, 44 in the AF and 49 in the PA, including captures and recaptures. Tapirs in the AF were captured both on the borders (*n*=17 individuals) as well as in the center of MDSP (*n*=18). Distances between the center and the borders varied from 10 to 15 km. Nineteen tapirs in the PA were not anesthetized but only visually evaluated (recapture followed by release).

Seven tapir deaths were recorded during the study: five in the AF (two were killed by jaguar, one was killed by a puma, and two died of unknown causes; Medici 2010) and two in the PA (one was killed by a puma, and one died of unknown causes; E.P.M. unpubl. data). Tapirs that died of unknown causes were old and in poor body condition before their death.

### Physical examination

Overall, PA tapirs were smaller than AF tapirs (Table 5). In 44 physical examinations in the AF, 77% of the tapirs were in good body condition. In 68 physical examinations in the PA (49 manipulations plus 19 recaptures followed by release), 75% of the tapirs were in good body condition (Table 6). No significant differences in tapir physical conditions were observed between sites (*P*=0.531) or between sexes (*P*=0.880). Adult tapirs had significantly more scars than subadults (*P*=0.005) and juveniles (*P*=0.014).

TABLE 5. Estimated body mass of wild lowland tapirs (*Tapirus terrestris*) in the Atlantic Forest (AF; 1996–2008) and Pantanal (PA; 2008–12), Brazil.

Sex	Age class	AF (n=44)			PA (n=68)		
		Mean (kg)	SD	n	Mean (kg)	SD	n
Female	Adult	230	32	25	221	11	20
	Subadult	170	7	2	190	0	8
	Juvenile	90	–	1	–	–	–
Male	Adult	205	38	11	210	18	16
	Subadult	189	17	4	190	0	15
	Juvenile	90	–	1	140	25	9

### Physiologic, hematologic, biochemical, and urinary parameters

Results for these parameters are presented in Tables 2 and 3, and Supplementary Material Tables S1–S3.

### Bacterial profile of anatomic cavities and dermal lesions

In the AF, 10 bacterial species were identified in anatomic cavities. *Staphylococcus aureus* and *Escherichia coli* were found in all anatomic cavities. In the PA, 26 bacteria were identified; *S. aureus* was the most prevalent species in anatomic cavities and present in 100% of sampled dermal lesions. Other bacteria found in at least five different cavities were *Acinetobacter* sp., Gram-positive bacilli, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and coagulase-negative staphylococci (Supplementary Material Table S4).

The calculation of similarity of bacteria species found in the AF and PA resulted in  $S_j=0.125$ , which means a low similarity between sites. Nevertheless, when comparing the prevalence of the four taxa found in both the AF and PA (*Enterobacter* sp., *E. coli*, *Staphylococcus* sp., and *S. aureus*) there were no significant differences between the two tapir populations.

### Infectious agents

The analyses of infectious agents allowed for important comparisons between the AF and PA tapir populations, as well as between sampling periods within each study site (annual disease prevalence;

Supplementary Material Tables S5 and S6).

Antibodies were detected for six agents: Bluetongue virus, eastern equine encephalitis (EEE) virus, western equine en-

TABLE 6. Results of physical evaluation of wild lowland tapirs (*Tapirus terrestris*) in the Atlantic Forest (AF; 1996–2008) and Pantanal (PA; 2008–12), Brazil.

Parameter	AF % (n=44)	PA % (n=68)
Body condition		
Good	77	75
Regular	16	23
Poor	7	2
Fur condition		
Altered pigmentation or physical hair condition	7	
Alopecia in the dorsal lumbar region		2
Skin		
Scars or recent wounds	34	47
Phlegmon		2
Eyes		
Unilateral corneal lesions		4
Senile halo	9	3
Bilateral lens opacity	5	2
Bilateral yellowish eye discharge		2
Periophthalmic glandular inflammation	5	
Genital		
Abnormal vaginal discharge	2	2
Vaginal mucosal hyperemia	2	
Dental conditions		
Fractures in incisors	5	

cephalomyelitis (WEE) virus, infectious bovine rhinotracheitis (IBR) virus, porcine parvovirus, and *Leptospira interrogans* (10 serovars: Autumnalis, Bratislava, Canicola, Copenhageni, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, and Pyrogenes).

The serologic screening for infectious agents revealed a high prevalence of exposure to *L. interrogans* in both sites, with 25% prevalence in the AF (95% CI: 12–38%) and 75% in the PA (95% CI: 66.1–83.9 for minimum estimated population size, and 63.7–86.3% for maximum estimated population size). Three *L. interrogans* serovars were found in the AF (Autumnalis, Hebdomadis, and Pomona), and eight in the PA (Bratislava, Canicola, Copenhageni, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona, and Pyrogenes). Pomona was the only serovar found in both populations. Eleven samples in the PA had antibody to three serovars. In the AF, antibody titers for *L. interrogans* Pomona were between 100 and 800, Autumnalis 400, and Hebdomadis 200. In the PA, considerably higher antibody titers were found: Pomona 100–3200, Bratislava 100–800, Icterohaemorrhagiae 100–800, Copenhageni 100–400, Canicola 100, Grippotyphosa 100, and Pyrogenes 800. Despite the high antibody titers, there were no clinical signs or laboratory results indicating disease.

The serologic response to bluetongue virus antigen resulted in a prevalence of 15.6% (95% CI: 4.7–26.5%) in the AF and 2.8% (95% CI: 0.3–12.6%) in the PA. Antibody to IBR virus was observed in 3.1% (95% CI: 0.06–15.7%) of the AF and 2.8% (95% CI: 0.3–12.3%) of the PA. Antibodies to EEE and WEE viruses were found only in the AF population: 18.7% (95% CI: 7–30.4%) and 3.1% (95% CI: 0.06–15.7%), respectively. Porcine parvovirus antibody was found only in the PA in 100% of evaluated tapirs (95% CI: 90.3–100%).

Significant differences in antibody prevalence between the study sites were

found for *L. interrogans* ( $P=0.000$ ), EEE virus ( $P=0.006$ ), and porcine parvovirus ( $P<0.000$ ). Nevertheless, there was no pattern in the distribution of positive results throughout the sampling period for most of the pathogens, except for *L. interrogans* and porcine parvovirus in the PA population.

## DISCUSSION

Physical examinations are one of the most relevant assessments of animal health and are complementary to laboratory findings (Deem et al. 2012; Singh et al. 2012; Travis et al. 2012). Physical abnormalities in this study were mostly explained by age (e.g., tooth wear, ocular senile halo) or social behavior (e.g., scars, wounds) rather than disease. Adult tapirs presented significantly more scars or wounds than subadults and juveniles. This likely reflects a buildup of scars over an individual's life, but could also suggest an increase in agonistic intraspecific (territoriality) or interspecific (predation) interactions in adults, or that adults survive predation better than younger individuals. Based on the physical assessment results and lack of clinical signs, both the AF and PA populations appear healthy.

Several hematologic and biochemical parameters were significantly different between the AF and PA tapir populations, as well as between wild (AF+PA) and captive tapirs (ISIS). Some differences may be explained by seasonal availability of resources in the wild, diet, competition, and reproductive state (Clauss et al. 2009; Jolly 2009). However, health conditions can also affect some parameters. Equines, one of the taxonomic groups closest to tapirs, experience variations in erythrocyte profile parameters due to dehydration, anemia, and acute stress (Duncan and Prasse 1986). Ectoparasite infestation can cause anemia in equines, a correlation that should be further evaluated in tapirs (Hernandez-Divers et al. 2005). Sampling methods, processing and storage of sam-

ples before analyses, laboratory protocols, and data analyses can also lead to different profiles for these parameters.

One of the most important differences between sites is that the Pantanal biome holds large populations of domestic and feral ungulates in proximity or contact with tapirs. The Pantanal is a natural mosaic of forest, open grasslands, and floodplains. Approximately 95% of the Pantanal is privately owned, and extensive cattle ranching is the main economic activity in the region. Domestic livestock and tapirs share the same space, which could explain the significantly higher diversity of bacteria in anatomic cavities and the overall higher prevalence of antibodies to pathogenic agents in PA tapirs. Likewise, the monocytosis in the PA could be an immunologic response to that. Monocytosis can be associated with chronic inflammatory conditions or exposure to certain pathogens (Almosny and Monteiro 2007).

Some of the isolated bacteria are considered opportunistic microorganisms that can cause disease in immune-depressed animals. Septicemia and enteritis caused by *Streptococcus* spp. have been reported for captive tapirs (Janssen 2003). Apical and mandibular abscesses in captive tapirs have been associated with *Streptococcus* spp. and *E. coli* (Janssen 2003). Both these bacteria were isolated in the PA tapir population, but no clinical signs were observed.

Urinary analysis showed pH and specific gravity results similar to those described for horses (Parrah et al. 2013). Red and white blood cell counts and the presence of bacteria in some urine samples could be associated with urinary infections or sample contamination (Parrah et al. 2013).

Serologic screening for infectious agents revealed a high prevalence of exposure to *L. interrogans* in both sites. Higher diversity of serovars and higher antibody titers were found in the PA. Leptospirosis is an acute or chronic zoonotic disease and its incidence is strongly associated with heavy rains,

standing water, and hot climate (Uhart et al. 2010). The Pantanal is a seasonally inundated floodplain, and its intrinsic environmental characteristics may be favorable to pathogens whose epidemiologic cycles depend on water. Pomona, the only serovar found in both sites, is commonly found in bovines and causes reproductive disorders. The most virulent serovar for domestic ungulates, Icterohaemorrhagiae, was only found in the PA. Five of 17 sampled Baird's tapirs in Costa Rica had antibody to Bratislava serovar (Hernandez-Divers et al. 2005).

Bluetongue virus was the second most prevalent infectious agent, found in both the AF and PA. Bluetongue is a vector-borne disease, and its prevalence can be related to environmental conditions that favor insect survival, such as high rainfall, temperature, and humidity (Tomich et al. 2009; Araújo et al. 2010). Infectious bovine rhinotracheitis virus was also found in both the AF and PA. Infectious bovine rhinotracheitis is caused by the bovine herpesvirus type 1 and is considered to be economically important in several parts of the world (Nogueira and Cruz 2007). Earlier investigations in the Pantanal detected antibody to IBR virus in 72.7% of capybaras and 37.5% of bovines sampled (Nogueira and Cruz 2007). Both species occupied the same area and sampled individuals appeared to be healthy. Antibody to EEE and WEE viruses were found only in the AF. Both viruses are extremely relevant for equines and humans, circulate among birds, and are transmitted by mosquitoes (Nogueira and Cruz 2007). Two of 17 Baird's tapirs sampled in Costa Rica were positive for antibody to EEE virus and two for WEE virus, and there was a particularly high prevalence of Venezuelan equine encephalitis virus with 14 positive individuals, 10 of those with high titers (60–640; Hernandez-Divers et al. 2005). There are no reports of clinical manifestations of equine encephalitis in tapirs in the wild or in captivity (Hernandez-Divers et al. 2005; Mangini et al. 2012).

Another important finding of this study was the high prevalence of porcine parvovirus in the PA. Porcine parvovirus was not found in the AF. Porcine parvovirus infection is highly prevalent in domestic pigs (*Sus scrofa*) in Brazil and is considered to be the main cause of reproductive problems in herds (Streck et al. 2011). Domestic pigs were introduced to the Pantanal 200 years ago and became feral during the Paraguay War. Today, feral pigs can be found throughout the region and could be potential reservoirs of several pathogens (Herrera et al. 2005; Ruiz-Fons et al. 2007). Domestic pigs are not found inside MDSP, although they are present near the park. Only peccaries are found inside MDSP. The presence of feral pigs could be responsible for the very high prevalence of porcine parvovirus infection in the PA tapir population. Nevertheless, there were no clinical signs of disease in PA tapirs.

Tapirs captured on the borders of MDSP often used the agriculture/pasture land matrix around MDSP when moving between the park and surrounding forest fragments (Medici 2010) and were closer to domestic animals than were tapirs in the center of the park. Cattle and horses in farms around MDSP presented a high prevalence of *Leptospira* spp. antibody (cattle, 41.2%; horses, 33.5%; Nava 2008). Serum antibodies to *L. interrogans*, bluetongue virus, EEE virus, and WEE virus were detected in tapirs in the borders as well as the center of MDSP. Antibody to IBR was found only in tapirs on the border, but it was detected on just one occasion. Tapirs exposed to *Leptospira* spp., bluetongue virus, and EEE, and WEE viruses as well as peccary species exposed to *Leptospira* spp. (white-lipped peccary, 29.5%; collared peccary, 10.5%; Nava 2008) in the center of MDSP indicate that distance from the borders of the park and consequently from domestic animals does not seem to affect prevalence.

The proximity between wildlife and domestic livestock can influence the epi-

demologic profile of tapir populations as well as create opportunities for pathogen transmission (Medici et al. 2007a). Although the AF and PA tapir populations were considered healthy, potential health issues caused by exposure to infectious agents cannot be disregarded. Wildlife health studies using an ecologic approach can suggest possible relationships between infectious agents, human, domestic animals, and wildlife facing different environmental characteristics. It will be important to monitor the influence of these interactions over time.

This long-term study provides the most extensive dataset of wild lowland tapir health information, including several new findings. The hematology, biochemistry, urinalysis, and microbiology results we report can be used as reference values for lowland tapirs in the wild and captivity. This article contributes to a better understanding of the potential relationship between the health of tapir populations and their environment and will better inform the development of conservation actions.

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