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HEALTH ASSESSMENT OF WILD LOWLAND TAPIRS (*TAPIRUS TERRESTRIS*) IN THE HIGHLY THREATENED CERRADO BIOME, BRAZIL

Renata Carolina Fernandes-Santos,[1,2,6,7] Emília Patrícia Medici,[1,2,3] Caroline Testa-José,[1] and Tatiane Micheletti[4,5,6]

[1] Lowland Tapir Conservation Initiative, Institute for Ecological Research, Campo Grande, Mato Grosso do Sul, 79046-150, Brazil
[2] International Union for Conservation of Nature, Species Survival Commission, Tapir Specialist Group, Campo Grande, Mato Grosso do Sul, 79046-150, Brazil
[3] School of Environmental Conservation and Sustainability, Nazaré Paulista, São Paulo, 12960-000, Brazil
[4] University of British Columbia, Faculty of Forestry, Department of Forest Resources Management, Vancouver, V6T1Z4, Canada
[5] Pacific Forestry Centre, Natural Resources Canada, Victoria, V8Z1M5, Canada
[6] Brazilian Institute for Conservation Medicine–TRÍADE, Curitiba, Paraná, 80250-060, Brazil
[7] Corresponding author (email: renatacfsantos@gmail.com)

ABSTRACT: Over 2 yr, we assessed the health of 35 lowland tapirs (*Tapirus terrestris*) in the Brazilian Cerrado (CE) biome, an area that is highly affected by human activities. This involved physical examinations, hematology and blood biochemistry, urinalysis, fecal parasitologic evaluation, microbial profiling of anatomic cavities and lesions, and serologic surveys for evidence of infectious agents. Research methods closely resembled those used in previous tapir health assessments in the Atlantic Forest (AF) and Pantanal (PA) biomes, allowing for a comparison among the three populations. Although not reaching statistical significance ($P>0.05$), tapirs from the CE exhibited poorer body and skin condition as compared to animals from the AF and PA. Furthermore, there were higher prevalences of dental problems and traumatic lesions as compared to those from the AF and PA. Eight of the 12 hematologic parameters evaluated and 17 of the 30 biochemical parameters differed significantly ($P<0.05$) between the tapirs from CE and those from the AF and PA. We isolated 24 different microbiologic strains from swabs of anatomic cavities and dermal lesions, of which five taxa had not previously been found in the AF or PA. We detected serum antibodies to *Leptospira interrogans*, bluetongue virus, and porcine parvovirus. Overall, our results suggested that tapirs from the CE exhibited more health abnormalities than tapirs in the AF and PA, possibly due to a greater exposure to environmental disturbances in the area.

Key words: Biochemistry, conservation, environmental disturbances, hematology, microbiology, One Health, Perissodactyla, serology.

INTRODUCTION

The lowland tapir (*Tapirus terrestris*), is the largest land mammal in Brazil and is listed by the International Union for Conservation of Nature as Vulnerable to Extinction (International Union for Conservation of Nature 2008). In the Cerrado (CE) biome (central-western Brazil), Brazil's epicenter of economic development, the species is listed as Endangered (Medici et al. 2018). Because tapirs are wide-ranging herbivores, they may cross human-impacted areas, resulting in exposure to a variety of threats that affect their long-term survival, such as the expansion of large-scale agriculture and cattle ranching, habitat loss and fragmentation, road kill, poaching, environmental contamination by pesticides and heavy metals, and increased exposure to domestic and feral animal diseases (Medici and Desbiez 2012).

The health of wild lowland tapir populations in several Brazilian biomes has been monitored since 1996 by a long-term research and conservation program carried out by the nongovernmental Institute for Ecological Research, the Lowland Tapir Conservation Initiative (LTCI). Reference values are available for physiologic, hematologic, and biochemical parameters of lowland tapir populations in the Atlantic Forest (AF; southern Brazil, 1996–2008) and Pantanal

(PA; central–western Brazil, 2008–12), where tapir populations were categorized as healthy (Medici et al. 2014).

Our main goal was to evaluate the health of lowland tapirs in the Brazilian CE and to compare findings with the previous results from the AF and PA biomes. The combination of ecologic and epidemiologic data in wildlife research builds a better understanding of ecosystem health as a whole and is critical to ensure the long-term survival of threatened species (Cunningham et al. 2017).

MATERIALS AND METHODS

Study site

The Cerrado covers 203,199,000 ha of the central Brazilian plateau and is the second largest of Brazil's major biomes, after the Amazon. It is the most extensive woodland savannah region in South America and is a global biodiversity hotspot. However, it is one of the most threatened and least protected biomes in Brazil (Sano et al. 2010).

The study site—a mosaic of different types of land use and ownership including farms, cattle ranches, landless settlements, and plantations—is located between the municipalities of Nova Alvorada do Sul and Nova Andradina, Mato Grosso do Sul State, Brazil (21°60′S, 53°83′W). It is approximately 220,000 ha and includes small fragments of natural Cerrado habitat (Cerradão fragments, gallery forests, and marshland) of about 12% of the study area, surrounded by areas highly impacted by human activities such as agriculture (particularly sugarcane, soybean, and corn), cattle-ranching, eucalyptus plantations, rural communities, and highways.

Capture and chemical restraint

Tapirs were captured by darting after physical restraint in box traps or pitfall traps or by darting from a distance (Medici 2010). Tapirs captured in box traps (n=28) and pitfalls (n=5) were anesthetized using a combination of butorphanol (0.17 mg/kg), medetomidine (0.012 mg/kg), and ketamine (0.7 mg/kg). Atropine (0.03 mg/kg) was added as needed. Atipamezole (0.04 mg/kg) and naltrexone (0.35 mg/kg) were used as reversal agents. Tapirs that were darted from a distance (n=2) were anesthetized using a combination of tiletamine-zolazepam (1.25 mg/kg), medetomidine (0.006 mg/kg), ketamine (0.6 mg/kg), and atropine (0.03 mg/kg), and midazolam (0.03 mg/kg) was administered 30 min later (Quse and Fernandes-Santos 2014). Anesthetics were administered intramuscularly using 3 cc or 5 cc darts, and anesthetic doses were calculated based on estimated body mass.

Handling and collection of biological samples

The procedures carried out during immobilization included the placement of a very high frequency-global positioning system telemetry collar on adults, subcutaneous insertion of a microchip, morphometric measurements, sex and age class determination, physical examination, and collection of blood, urine, feces, ectoparasites, skin biopsy, fur, milk, and swabs of dermal lesions and the oral, nasal, auricular, ocular, anal, vaginal, urethral, and preputial areas (Medici et al. 2014; Quse and Fernandes-Santos 2014).

Physiologic parameters were monitored every 10 min throughout anesthesia and included respiratory rate, heart rate, and blood oxygen saturation (through pulse oximetry, Oxy9Vet-Plus®, Bionet America, Tustin, California, USA), body temperature (using a digital thermometer rectally), and noninvasive systolic blood pressure (through a portable vascular Doppler, Mini Dopplex® with a 10 MHz probe, Cardiology Shop, Boston, Massachusetts, USA). The mean duration of anesthesia was 69 (±20) min.

Physical examination included overall body condition (good, average, or poor), based on criteria used by Clauss et al. (2009) and Pérez-Flores et al. (2016), skin condition and integrity (presence of scars or wounds, alopecia, alterations in pigmentation), fur condition, examination of anatomic cavities (ocular, nasal, auricular, oral including dental evaluation), anus, vagina, urethra, and preputial area, palpation and thoracic and abdominal auscultation, evaluation of musculoskeletal integrity and mobility, condition of nails and foot pads, and reproductive examination (in females, vaginal inspection, evaluation of mammary glands, presence of milk, evidence of reproductive activity; in males, examination of penis and testes). Individuals were categorized into three age classes based on dentition, tooth wear, erosion of nails, and appearance of foot pads. Age classes were juvenile (12–17 mo), subadult (18–47 mo), and adult (>48 mo).

About 60 mL of blood was collected within 20 min of immobilization through venipuncture of the medial saphenous or cephalic veins using vacuum sampling tubes (plain and with ethylenediaminetetraacetic acid). In cases of spontaneous urination during anesthesia, urine samples were collected in sterile tubes. Fecal samples were collected when captured tapirs spontaneously defecated inside the traps. Sterile swabs of anatomic areas and active wounds were placed in Stuart transport medium. All samples were

placed in a portable cooler with ice and immediately transported from the capture site to a field laboratory where they were preprocessed and stored for later laboratory analysis.

Laboratory examinations

Blood in ethylenediaminetetraacetic acid was processed within 24 h of collection in the field laboratory. Complete blood counts, blood smear evaluations (Diff Quick stain, Laborclin®, Pinhais, Paraná, Brazil), and differential leukocyte counts were performed manually, always by the same veterinarian. Morphologic alterations in blood cells and presence or absence of hemoparasites were documented. Blood samples without anticoagulant were centrifuged within 12 h of collection, and serum aliquots frozen at –20 C for up to 15 d until transportation to a laboratory. Serum biochemical evaluation was carried out in a human diagnostic laboratory using an automated chemistry analyzer (Dimension®, Siemens, Erlangen, Germany).

Urine samples were analyzed in the field using urine test strips (Urofita®, Prodimol, Belo Horizonte, Minas Gerais, Brazil). Fecal samples were analyzed for endoparasites by centrifugal flotation in supersaturated sucrose solution (qualitative and semiquantitative analyses). Swabs were refrigerated and transported to a laboratory within 72 h of collection and cultured for aerobic and anaerobic bacteria.

We conducted serologic analyses to survey for antibodies to 14 infectious agents (viral and bacterial) relevant to tapir health and well known to affect local domestic livestock or as important zoonoses. Serologic tests were carried out in a reference laboratory in Brazil (Instituto Biológico de São Paulo, São Paulo).

Data analysis

To allow for comparison of results with the previous tapir health assessments in the AF and PA, we performed the same analyses as did Medici et al. (2014). Hematologic and biochemical variables were tested for normality (Shapiro-Wilk test) and variance homogeneity (Levene's test) to be compared by groups. The groups were hierarchically designed as study site>sex. Only the variables that were not significantly different for each group (sex) were compared on the next level (study site). For data with normal distribution and homogeneity of variances before or after transformation (natural logarithm), a parametric analysis of variance or Student's *t*-test was used. When normality could not be achieved through transformation, nonparametric Kruskal-Wallis tests or Mann-Whitney *U* tests were applied. Alpha was set to 0.05. 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.

While the results of physical examination, parasitologic evaluation, and physiologic and urinalysis parameters were tabulated and presented descriptively, the relative prevalence of microbiologic strains in the anatomic cavities and dermal lesions was statistically analyzed for each cavity and for the entire sampled population. The chi-square test was used to compare the prevalence of microbiologic strains among study sites (CE, AF, and PA). The diversity of microorganisms and the similarity among study sites were measured using the Jaccard similarity coefficient (Sj; Magurran 2004), where Sj=1 represented study sites with similar microbiologic profile, and an Sj=0 represented those with different microbiologic profiles (Wilson and Mohler 1983).

To determine the percentage of the population sampled in the serologic survey of infectious agents, the tapir population in the study area was estimated to be between 550 and 1,430 individuals, based on preliminary density estimates between 0.0025 and 0.0065/ha. The sampling prevalence for infectious agents was calculated as the proportion of tapirs with positive antibody responses, and the 95% confidence interval (CI) were calculated as a simple random sampling (Thrusfield 2007). Chi-square tests were used to compare prevalence data by study site.

RESULTS

We captured and sampled 35 lowland tapirs (21 females, 14 males; Table 1) between September 2015 and September 2017, which represents 2.5–6.4% of the tapir population in the study site. Three tapirs (one adult female, one juvenile female, and one adult male) were killed by collision with vehicles, and one adult female died due to an unknown cause during the study.

Physical examination

Physiological parameters monitoring during anesthesia demonstrated lower values than those described by Medici et al. (2014) for heart and respiratory rates (Table 2). However, differences in the anesthetic protocols and in the health status of individuals should be considered.

TABLE 1. Age classes and mean estimated body mass (kg) of 35 wild lowland tapirs (*Tapirus terrestris*) captured in the Cerrado, Mato Grosso do Sul State, Brazil (2015–17). No juvenile male tapirs were captured.

Sex	Age class	*n*	Mean (SD) body weight (kg)
Female	Adult	15	201 (20)
	Subadult	2	180 (0)
	Juvenile	4	95 (11)
Male	Adult	8	190 (10)
	Subadult	6	155 (28)

Physical examinations showed notable alterations (Table 3), particularly in tapirs' dentition, including tooth loss, fractures, periodontitis, and gingival retraction in 57% (20/35) of the captured individuals. Two males lacked upper and lower incisors. No dental problems were observed in tapirs from the PA, and only 4.5% of AF tapirs presented fractures of incisors. Nine percent of tapirs from the CE were classified in poor body condition, compared to 6.8% in AF and 1.5% in PA, but the difference was not statistically significant, and there was no positive correlation with sex, age or season. While poor or average body conditions were observed in 29% (10/35) of tapirs evaluated, skin condition was classified as average or poor in 26% (9/35) of the individuals, scars were observed in 57% (20/35), and fresh wounds in 34% (12/35).

TABLE 2. Physiologic parameters of 33 wild lowland tapirs (*Tapirus terrestris*) under anesthesia using a combination of butorphanol (0.17 mg/kg), medetomidine (0.012 mg/kg), and ketamine (0.7 mg/kg) in the Cerrado, Mato Grosso do Sul State, Brazil (2015–17). Tapirs in the Cerrado do not represent a healthy wild tapir population, and results should not be used as reference values for the species.

Parameter	Unit	*n*	Mean	SD
Heart rate	bpm	33	56	15
Respiratory rate	bpm	33	23	10
Blood oxygen saturation	%	32	90	8
Body temperature	C	32	36.4	0.9
Systolic blood pressure	mmHg	7	104	12

TABLE 3. Results of physical evaluation of 35 wild lowland tapirs (*Tapirus terrestris*) in the Cerrado, Mato Grosso do Sul State, Brazil (2015–17).

Condition	% Presence	Category or alteration
Body	71	Good
	20	Average
	9	Poor
Skin	74	Good
	23	Average
	3	Poor
Skin integrity	57	Presence of scars
	34	Presence of recent wounds
Fur	11	Altered pigmentation
Eye	11	Bilateral yellowish mucous conjunctival discharge
Dental	57	Tooth loss, fractures, periodontitis, or gingival retraction
Other findings	3	Abnormal respiratory discharge
	3	Umbilical hernia
	3	Absence of one ear pinna
	6	Penis injury
	3	Edema and inflammation of the lip

Wounds were mostly associated with traumatic events and inflammatory processes such as nodules and pustules (Murphy et al. 2006). One adult male exhibited a severe penis injury (an extensive inflammatory process with purulent exudate and clear disablement), and one male was missing its right pinna. Altered pigmentation of the hair was observed in 11% (4/35) of tapirs. Ophthalmic examination revealed bilateral yellowish mucous conjunctival discharge in 11% (4/35) of individuals.

Hematologic and biochemical parameters

Morphological alterations in red blood cells, including the presence of Howell-Jolly bodies (63%; 22/35), rouleaux (11%; 4/35), anisocytosis (3%; 1/35), and hypochromia (3%; 1/35), were observed in some individuals. Alterations in white blood cells included presence of toxic neutrophils (29%; 10/35) and reactive lymphocytes (80%; 28/35). An abnormally high

TABLE 4. Hematologic parameters of 35 wild lowland tapirs (*Tapirus terrestris*) in the Cerrado (CE), Mato Grosso do Sul State, Brazil (2015–17). Tapirs in the CE do not represent a healthy wild tapir population, and results should not be used as reference values for the species.

Analyte	Unit	*n*	Mean	Min	Q1[a]	Median	Q3[a]	Max	SD	SE	*P* value[b] CE vs. AF[c]	*P* value[b] CE vs. PA[d]
Red blood cell count	10^{12}/L	35	6.50	4.19	5.57	6.35	7.24	9.73	1.40	0.24	<0.001	0.041
Packed cell volume	L/L	35	0.29	0.19	0.27	0.29	0.32	0.36	0.04	0.01	0.105	<0.001
Mean corpuscular volume	fL	35	46.43	31.05	38.89	45.57	53.27	72.69	9.45	1.60	<0.001	<0.001
White blood cell count	10^9/L	35	14.38	6.60	9.38	14.25	18.85	28.35	5.61	0.95	<0.001	<0.001
Eosinophils	10^9/L	35	0.39	0	0	0.18	0.41	1.85	0.56	0.10	0.413	
Basophils	10^9/L	35	0.03	0	0	0	0	0.38	0.08	0.01	0.278	
Lymphocytes	10^9/L	35	2.97	1.19	1.90	2.95	3.82	7.31	1.33	0.22	0.124	
Reactive lymphocytes	10^9/L	35	0.62	0	0.14	0.39	0.87	3.67	0.74	0.12	NA[e]	
Monocytes	10^9/L	35	0.66	0.00	0.40	0.56	0.78	2.46	0.47	0.08	<0.001	<0.001
Band neutrophils	10^9/L	35	0.38	0	0	0.08	0.34	2.78	0.65	0.11	0.008	0.285
Segmented neutrophils	10^9/L	35	9.96	3.72	5.95	9.15	12.55	25.80	5.07	0.86	<0.001	<0.001
Total neutrophils	10^9/L	35	10.33	3.80	5.95	9.15	12.85	25.80	5.33	0.90	0.001	<0.001

[a] Q1 = lower quartile; Q3 = upper quartile.

[b] The *P* value was compared separately for CE and the other two biomes only if it was significant after a three-way comparison. In these cases, the *P* value was determined by the Dunn test for nonparametric data and by the Tukey HSD test for parametric data. Otherwise, the three-way *P* value is reported.

[c] AF = Atlantic Forest.

[d] PA = Pantanal.

[e] NA = not applicable, detected only in CE.

number of immature white blood cells was observed in three individuals. No direct correlations with clinical signs of disease were observed in the affected individuals. Hemoparasites were not detected in blood smears.

Statistical comparisons between data from lowland tapirs sampled in the CE and lowland tapirs from the PA and AF showed significant difference ($P<0.05$) for eight of the 12 hematologic parameters evaluated (Table 4) and for 17 of the 30 biochemical parameters (Table 5). Mean concentrations of enzymes associated with liver function (such as aspartate aminotransferase and gamma glutamyl transferase) were significantly higher than values observed in tapirs captured in the AF and PA.

Urinalysis and parasitologic evaluation

In general, urinalysis showed no findings that differed significantly from tapirs from the PA and AF, and parameters evaluated were considered normal (Parrah et al. 2013; Medici et al. 2014; Table 6). We found low levels (1–5 eggs on the blade) of ascarid eggs in 50% and strongylid eggs in 8% of fecal samples from 12 tapirs we examined. Parasites were not identified to species level.

Microbiologic profile of anatomic areas and dermal lesions

We isolated 24 different microbiologic strains from swabs of anatomic areas and dermal lesions (Table 7). *Serratia marcescens*, *Staphylococcus aureus*, and *Staphylococcus intermedius* were the most prevalent bacteria in the CE population. The eye contained the most diverse microbiota (13 different strains), followed by the auricular (12 strains) and anal areas (11 strains). We isolated five bacterial species (*Acinetobacter lwoffii*, *Burkholderia cepacia*, *Enterococcus faecalis*, *Morganella morganii*, and *Streptococcus viridans*) from CE tapirs that were not found in animals from the AF and PA (Medici et al. 2014).

The similarity index of microbiologic species found in the CE, PA, and AF was 0.45 for

TABLE 5. Biochemical parameters of 35 wild lowland tapirs (*Tapirus terrestris*) in the Cerrado (CE), Mato Grosso do Sul State, Brazil (2015–17). Tapirs in the CE do not represent a healthy wild tapir population, and results should not be used as reference values for the species.

										P value[b]	
Analyte	Unit	Mean	Minimum	Q1[a]	Median	Q3[a]	Maximum	SD	SE	CE vs. AF[c]	CE vs. PA[d]
Alanine aminotransferase	U/L	13.31	4.00	10.00	12.00	17.50	28.00	6.19	1.05	0.037	<0.001
Aspartate aminotransferase	U/L	118.83	41.00	79.50	102.00	130.50	344.00	62.65	10.59	<0.001	<0.001
Gamma glutamyl transferase	U/L	19.43	3.00	15.00	19.00	23.00	36.00	7.12	1.20	<0.001	0.008
Blood urea nitrogen	mmol/L	6.22	1.79	4.82	6.07	7.50	10.35	2.02	0.34	0.054	
Uric acid	µmol/L	12.07	0	5.95	11.90	11.90	53.53	12.28	2.08	0.111	
Creatinine	µmol/L	83.10	53.04	79.56	79.56	88.40	114.92	12.54	2.12	<0.001	<0.001
Creatinine phosphokinase	U/L	602.91	3.00	146.00	213.00	699.50	3232.00	791.74	133.83	0.219	<0.001
Alkaline phosphatase	U/L	18.69	3.00	14.50	16.00	22.50	56.00	10.40	1.76	0.047	0.006
Lactate dehydrogenase	U/L	717.91	215.00	566.50	633.00	787.50	1532.00	286.61	48.45	NA[e]	
Glucose	mmol/L	7.08	3.11	5.77	7.10	8.16	10.82	1.98	0.33	0.970	0.049
Total cholesterol	mmol/L	3.40	1.58	2.81	3.16	4.06	6.01	0.92	0.16	0.715	
HDL cholesterol[f]	mmol/L	2.16	0.85	1.91	2.10	2.45	3.34	0.53	0.09	0.081	
LDL cholesterol[f]	mmol/L	1.11	0.18	0.74	1.04	1.50	2.51	0.54	0.09	NA[g]	0.150
VLDL cholesterol[f]	mmol/L	0.13	0.03	0.08	0.10	0.16	0.34	0.08	0.01	NA[g]	0.002
Triglyceride	mmol/L	0.73	0.03	0.16	0.25	0.35	16.54	2.76	0.47	<0.001	<0.001
Total protein	g/L	69.46	54.00	65.50	69.00	75.00	83.00	7.56	1.28	0.125	<0.001
Albumin	g/L	17.46	8.00	16.00	17.00	19.00	35.00	4.57	0.77	<0.001	0.340
Globulin	g/L	52.03	34.00	48.00	51.00	56.50	66.00	6.58	1.11	0.187	0.015
Albumin:Globulin	(ratio)	0.34	0.17	0.28	0.33	0.37	1.03	0.14	0.02	0.158	
Cholinesterase	U/L	267.17	143.00	214.00	249.00	287.00	955.00	131.17	22.17	0.130	
Total bilirubin	µmol/L	6.83	2.74	3.93	5.13	7.36	21.03	4.11	0.70	<0.001	0.032
Direct bilirubin	µmol/L	1.51	0.34	0.86	1.71	1.71	4.10	0.72	0.12	<0.001	<0.001
Indirect bilirubin	µmol/L	5.33	1.71	2.91	3.42	6.42	18.47	3.79	0.64	0.001	0.298
Magnesium	mmol/L	0.65	0.37	0.53	0.62	0.70	1.07	0.17	0.03	0.523	
Sodium	mmol/L	139.20	124	135	140	143	155	6.55	1.11	<0.001	0.002
Potassium	mmol/L	3.39	2.20	3.05	3.50	3.65	4.50	0.55	0.09	0.132	
Calcium	mmol/L	2.33	2.00	2.24	2.33	2.44	2.65	0.17	0.03	0.122	
Phosphorus	mmol/L	1.02	0.48	0.80	0.94	1.24	1.81	0.35	0.06	0.119	
Chloride	mmol/L	103.97	95.00	99.50	103.00	107.00	128.00	7.34	1.24	0.030	0.005
Iron	µmol/L	10.62	2.69	8.06	9.85	13.25	22.20	4.16	0.70	0.011	<0.001

[a] Q1 = lower quartile. Q3 = upper quartile.

[b] The *P* value was compared separately for CE and the other two biomes only if it was significant after a three-way comparison. In these cases, the *P* value was determined by the Dunn test for nonparametric data and by the Tukey HSD test for parametric data. Otherwise, the three-way *P* value is reported.

[c] AF = Atlantic Forest.

[d] PA = Pantanal.

[e] NA = not applicable; detected only in CE.

[f] HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.

[g] NA = not applicable; no samples available for this comparison.

TABLE 6. Urinalysis analytes of five wild lowland tapirs (*Tapirus terrestris*) in the Cerrado, Mato Grosso do Sul State, Brazil (2015–17).

Analyte	Result	*n*
Specific gravity	1.012 (11)[a]	5
pH	6.1 (0.7)[a]	5
Color	Light yellow	5
Presence of protein	Positive	2
	Negative	3
Glucose	Negative	5
Ketone bodies	Positive	1
	Negative	4
Biliary pigments	Negative	5
Hemoglobin	Negative	5
Bilirubin	Negative	5
Nitrite	Negative	5
Urobilinogen mg/dL	Normal	4
	Negative	1
Leukocytes/mL	Negative	3
	About 25	2
Erythrocytes/mL	Negative	5
Hyaline casts	Negative	5
Crystals	Negative	5
Bacteria	Negative	5

[a] Mean (SD).

CE and PA (meaning a lower microbiota similarity between these sites) and 0.90 for CE and AF (indicating greater microbiota similarity). Nevertheless, there were no significant differences among the only three bacterial species (*Enterobacter* sp., *Escherichia coli*, and *S. aureus*) found in all three tapir populations.

Antibodies to infectious agents

Antibodies were detected to three of the 14 infectious agents tested for (Table 8). The serologic screening revealed a high prevalence of exposure, resulting in 60% (95% CI: 42–76%) with detectable antibody to *Leptospira interrogans*, 91% (95% CI: 77–98%) to bluetongue virus, and 97% (95% CI: 85–100%) to porcine parvovirus. We found significant differences in antibody prevalence between study sites for *L. interrogans* (only when compared to AF, $P=0.019$), bluetongue virus (PA $P<0.001$; AF $P<0.001$), and porcine parvovirus (only when compared to AF, $P<0.001$). Positive antibody titers were observed for two of the 26 serovars of *L. interrogans* tested for, including Pomona (in 19 individuals, with titers ranging between 100 and 3200) and Grippotyphosa (in two individuals, with titers of 100 and 200). Antibody titers for porcine parvovirus ranged between 2 and 256.

DISCUSSION

This study provided a comprehensive assessment of the health status of wild lowland tapirs in a highly threatened habitat, including comparisons among three Brazilian biomes under different levels of environmental disturbance. Sampling methods, laboratory protocols, and data analysis followed those of Medici et al. (2014) in the PA and AF biomes; significant differences were found.

Abnormalities found during physical examinations were likely environmentally related. The poor body condition may have reflected differences in nutritional status, possibly associated with dental abnormalities. Dental lesions are a frequent problem for tapirs, occurring in captive and wild individuals, and are usually associated with trauma, increasing age, bacterial presence, or food resources (Da Silva et al. 2011; Tjørnelund et al. 2015). The diet of tapirs in the CE is characterized mainly by leaves, stems, and small fruits (Talamoni and Assis 2009). However, tapirs can shift their foraging strategy depending on the availability of different food items (Medici 2010). The quality and availability of food resources in the fragmented CE study site may not have been suitable to maintain the dental and nutritional health of tapirs. In addition, the trauma observed in CE tapirs were different from those seen in healthy tapirs in PA and AF. Some injuries (such as penis lesions, edema and inflammation of the lip, and loss of a pinna) might impact normal behaviors such as reproduction, feeding, and social interactions.

Several hematologic and biochemical parameters were significantly different among tapirs in the CE, PA, and AF. Some differences may be explained by physiologic

TABLE 7. Microbiologic strains isolated from anatomic cavities and dermal lesions and their prevalence by cavity type and in the population of 35 wild lowland tapirs (*Tapirus terrestris*) in the Cerrado (CE), Mato Grosso do Sul State, Brazil (2015–17).

Microbiologic strain	Sampling prevalence: % (95% confidence interval)										*P* value[a]	
	Oral	Nasal	Auricular	Anal	Vaginal	Urethral	Preputial	Ocular	Dermal lesions	Total	CE vs. AF[b]	CE vs. PA[c]
Frequency[d]	8/35	9/34	12/34	11/35	4/21	3/13	7/14	13/35	6/6	24/35		
Acinetobacter sp.	3 (0–14)	0	6 (1–19)	3 (0–14)	0	0	7 (0–34)	0	0	2 (1–5)	0.984	1.000
Acinetobacter lwoffii	0	3 (0–14)	3 (0–14)	0	0	0	0	0	0	1 (0–3)	NA[e]	
Bacillus sp.	3 (0–14)	0	0	0	0	0	0	0	0	0 (0–2)	1.000	0.003
Burkholderia cepacia	0	0	0	0	0	0	0	3 (0–14)	0	0 (0–2)	NA[e]	
Candida sp. (not *C. albicans*)	0	0	0	0	0	0	0	5 (1–18)	8 (0–38)	1 (0–3)	1.000	1.000
Enterobacter aerogenes	3 (0–14)	3 (0–14)	3 (0–14)	0	0	0	0	8 (2–22)	0	2 (1–5)	0.552	1.000
Enterobacter agglomerans	0	6 (1–19)	11 (3–26)	0	0	0	0	13 (4–29)	0	4 (2–8)	0.116	0.354
Enterobacter cloacae	3 (0–14)	0	11 (3–26)	0	0	0	7 (0–34)	5 (1–18)	0	3 (1–6)	0.336	0.034
Enterobacter sp.	0	0	0	0	0	0	7 (0–34)	0	0	0 (0–2)	0.657	1.000
Enterococcus sp.	0	0	0	13 (4–28)	0	0	0	0	0	2 (1–5)	0.984	0.654
Enterococcus faecalis	0	0	0	3 (0–14)	0	0	0	0	0	0 (0–2)	NA[e]	
Escherichia coli	0	3 (0–14)	6 (1–19)	3 (0–14)	0	7 (0–34)	7 (0–34)	3 (0–14)	0	3 (1–6)	<0.001	0.348
Klebsiella oxytoca	0	0	0	0	0	0	0	0	17 (2–48)	1 (0–3)	1.000	1.000
Klebsiella pneumoniae	0	3 (0–14)	3 (0–14)	3 (0–14)	5 (0–24)	0	7 (0–34)	3 (0–14)	0	2 (1–5)	0.552	0.648
Morganella morganii	0	0	0	3 (0–14)	0	0	0	0	0	0 (0–2)	NA[e]	
Pseudomonas aeruginosa	16 (6–31)	3 (0–14)	8 (2–22)	0	5 (0–24)	0	0	3 (0–14)	0	5 (2–8)	0.063	0.762
Serratia marcescens	0	17 (6–33)	11 (3–26)	5 (1–18)	9 (1–30)	21 (5–51)	29 (8–58)	8 (2–22)	0	10 (6–14)	<0.001	0.054
Staphylococcus aureus	13 (4–28)	11 (3–26)	11 (3–26)	5 (1–18)	0	0	7 (0–34)	8 (2–22)	17 (2–48)	8 (5–13)	<0.001	0.534
Staphylococcus intermedius	8 (2–21)	11 (3–26)	8 (2–22)	8 (2–21)	0	7 (0–34)	0	3 (0–14)	17 (2–48)	7 (4–11)	0.007	<0.001
Coagulase-negative staphylococci	0	0	3 (0–14)	0	0	0	0	0	0	0 (0–2)	1.000	0.003
Stenotrophomonas maltophilia	0	0	0	0	0	0	0	0	8 (0–38)	0 (0–2)	1.000	1.000
Streptococcus agalactiae (Group B)	0	0	0	8 (2–21)	0	0	0	0	0	1 (0–3)	1.000	0.687
Streptococcus viridans	0	0	0	0	0	0	0	3 (0–14)	0	0 (0–2)	NA[e]	
Beta-hemolytic streptococci	3 (0–14)	0	0	3 (0–14)	9 (1–30)	0	0	3 (0-14)	25 (5-57)	3 (1-6)	0.336	0.327

[a] The *P* value was compared separately for CE and the other two biomes only if it was significant after a three-way comparison. In these cases, the *P* value was determined by the Dunn test for non-parametric data and by the Tukey HSD test for parametric data. Otherwise, the three-way *P* value is reported.

[b] AF = Atlantic Forest.

[c] PA = Pantanal.

[d] *n* isolated/*n* tapirs tested.

[e] NA = not applicable; strains isolated only in the CE study site.

TABLE 8. Prevalence of antibodies to infectious agents and diagnostic method applied in the serosurvey of 35 wild lowland tapirs (*Tapirus terrestris*) in the Cerrado (CE), Mato Grosso do Sul State, Brazil (2015–17).

Category	Infectious agent	*N*	Percent seroprevalence (95% confidence interval)	Diagnostic method[a]	*P* value[b]	
					CE vs. AF[c]	CE vs. PA[d]
Viral	Bovine viral diarrhea virus	35	0	ELISA	1.000	
	Foot and mouth disease virus	35	0	AGID	1.000	
	Equine infectious anemia virus	12	0	AGID	1.000	
	Bovine leukemia virus	35	0	AGID	1.000	
	Eastern equine encephalitis virus	35	0	Serum neutralization in VERO cells	<0.001	1.000
	Western equine encephalitis virus	35	0	Serum neutralization in VERO cells	1.000	
	Bluetongue virus	35	91 (76.9–98.2)	AGID	<0.001	<0.001
	Infectious bovine rhinotracheitis virus	35	0	Serum neutralization in MDBK cells and AGID	0.964	1.000
	Pseudorabies virus (Suid herpesvirus type 1)	35	0	Serum neutralization in VERO cells	1.000	
	Vesicular stomatitis virus	35	0	Serum neutralization in VERO cells	1.000	
	Porcine parvovirus[a,d]	35	97 (85.1–99.9)	Hemagglutination inhibition	<0.001	1.000
	Classic swine fever virus	21	0	ELISA	NA[e]	
Bacterial	*Leptospira interrogans* (26 serovars)	35	60 (42.1–76.1)	Microscopic agglutination test	0.019	0.957
	Brucella abortus	35	0	Plate serum agglutination, Tube serum agglutination	1.000	

[a] ELISA = enzyme-linked immunosorbent assay; AGID = agar gel immunodiffusion; VERO = African green monkey kidney cell line; MDBK = Madin and Darby bovine kidney cells.

[b] The *P* value was compared separately for CE and the other two biomes only if it was significant after a three-way comparison. In these cases, the *P* value was determined by the Dunn test for non-parametric data and by the Tukey HSD test for parametric data. Otherwise, the three-way *P* value is reported.

[c] AF = Atlantic Forest.

[d] PA = Pantanal.

[e] NA = not applicable; tested only in CE.

and reproductive status, nutrition, or the stress of capture and physical restraint (Clauss et al. 2009; Hall et al. 2014). Healthy horses often display rouleaux formation and occasional Howell-Jolly bodies (Grondin and Dewitt 2010), and the close taxonomic relationship between horses and tapirs could explain these findings. Nevertheless, hematologic and biochemical parameters are also highly affected by health issues, and interpreting laboratory results requires a systematic evaluation of all factors involved. The main hematologic findings in the CE animals included significantly higher leukocyte, monocyte, segmented and total neutrophil counts, in comparison with the AF and PA tapirs. An increase in white blood cells can be associated with inflammatory conditions or stress or may indicate infection or exposure to certain pathogens (Almosny and Monteiro 2007). In addition, the presence of reactive lymphocytes was observed in 80% of individuals. Reactive lymphocytes are seen in peripheral blood as a response to systemic antigenic stimulation

secondary to both infectious and noninfectious disorders (Valenciano et al. 2014). Regarding biochemical parameters, aspartate aminotransferase and gamma glutamyl transferase have high specificity for liver disease in equines, and their serum increase is a usual finding in significant hepatopathy in horses (Durham et al. 2003; Thrall et al. 2014; Sohail et al. 2017). Mean concentrations of enzymes associated with liver function (such as aspartate aminotransferase and gamma glutamyl transferase) were significantly higher than values observed in tapirs captured in the AF and PA. Several other biochemical parameters were significantly different among the biomes. However, the correlation was not clear, and analytes with mean values in between AF and PA data were considered normal. The physiologic function of some of these parameters is not specific and alterations may be due to numerous other processes.

Endoparasite presence has not been associated with clinical signs in wild tapirs (Mangini et al. 2012; Medici et al. 2014) but has been observed in captive tapirs (Quse and Fernandes-Santos 2014). Further investigation is needed to identify endoparasites to the species level and to better understand the parasite-host balance in wild tapirs.

We isolated the bacteria *A. lwoffii*, *B. cepacia*, *E. faecalis*, *M. morganii*, and *S. viridans* in swabs of anatomic areas of tapirs. Although all of these are recognized as normal flora of healthy individuals, some bacteria could represent a potential opportunistic pathogen in hosts with impaired immune systems. *Acinetobacter lwoffii*, for example, has been identified as a cause of septicemia, pneumonia, meningitis, acute gastroenteritis, urinary tract infections, and skin and wound infections in humans (Regalado et al. 2009). In tapirs, bacteria such as *Streptococcus* spp., *Klebsiella* spp., and *E. coli* have been reported as causing respiratory disease, septicemia, enteritis, and apical and mandibular abscesses (Janssen 2003; Klimes et al. 2013). *Staphylococcus aureus* was previously isolated in 100% of the dermal lesions evaluated in the PA tapir population (Medici et al. 2014). However, none of the clinical signs observed in captured tapirs in the CE could be directly correlated with the presence of one or more of the isolated microorganisms.

Our serologic survey for infectious agents revealed a high prevalence of antibodies to *L. interrogans*, bluetongue virus, and porcine parvovirus. Serum antibody titers against *L. interrogans* in the absence of clinical signs have been reported in wild tapirs (Hernández-Divers et al. 2005; Mangini et al. 2012; Medici et al. 2014). Antibodies to *L. interrogans* were detected in tapirs in the AF and PA sites, with higher prevalence (75%) in the PA (Medici et al. 2014). *Leptospira interrogans* serovar Pomona was the only one found in all tapir populations (CE, PA, and AF). A previous study of 10 wild lowland tapirs in a protected area in the Cerrado between 2000 and 2002 revealed no detectable antibody to *L. interrogans* (Furtado et al. 2010). Leptospirosis is a global zoonotic disease affecting a wide range of animal species (including humans) and may be a threat to both captive and wild tapirs. In equines, leptospirosis causes significant changes in hematologic and other laboratory findings, including leukocytosis, neutrophilia, monocytosis, and elevated levels of serum enzymes of liver function (such as aspartate aminotransferase and gamma glutamyl transferase; Tonin et al. 2012; Sohail et al. 2017). All of these alterations were detected in assessed individuals. However, leptospirosis is a complex disease, and the diagnosis must be meticulously and individually assessed. Clinical signs of fever, anorexia, conjunctival suffusions, petechial hemorrhage on the mucosa, and hematuria, combined with hemolytic anemia, liver and renal failure, are frequent findings for equine leptospirosis (Sohail et al. 2017) but were not observed in captured tapirs in the CE.

Bluetongue virus has been reported in domestic ruminants in several regions of Brazil, including in Mato Grosso do Sul State (Tomich et al. 2009). As a vector-borne disease, the high prevalence of exposure of tapirs to bluetongue that we found was probably related to environmental conditions that favored insect survival and virus spread (Epstein 2002; Tomich et al. 2009; Araújo

Júnior et al. 2010; Campbell-Lendrum et al. 2015). Bluetongue virus usually affects domestic and wild ruminants and is typically asymptomatic but can lead to acute disease with high morbidity and mortality (Yavari et al. 2018). No clinical or laboratory findings indicating bluetongue disease were observed in our study. Antibodies were also detected in the AF and PA sites (Medici et al. 2014).

Porcine parvovirus antibodies were detected in 97% of tapirs captured in the CE, with no observed clinical manifestations. A high antibody prevalence was also observed in the PA tapir population (100%; 95% CI: 90–100%), and it was suggested that feral pigs (*Sus scrofa*) could be reservoirs of the virus (Medici et al. 2014). In the Cerrado, invasive boars have been causing significant environmental degradation (Pedrosa et al. 2015), and we hypothesize that they may be reservoirs of the virus in this biome.

Although the studies were not performed simultaneously, our results suggest that tapirs living in the CE are not as healthy as tapirs in the AF and PA. Significant clinical and laboratory findings support this conclusion, and several hypotheses are proposed. The values presented in this manuscript for physiologic, hematologic, and biochemical parameters from tapirs in the CE do not represent a healthy wild tapir population and should not be used as reference values for the species.

Environmental contamination by pesticides and heavy metals widely used on monoculture plantations is an important hazard in the CE. The LTCI has evaluated residual toxic substances in the blood and tissues of captured and road-killed CE tapirs. Pesticides commonly associated with physiologic disorders (e.g., liver failure) have been detected (Santos et al. 2017). In cases of chronic intoxication, usually due to repeated exposure to the substance over prolonged periods (months or years), the adverse health effects may include immunologic, hematologic, hepatic, and neurologic disorders, congenital malformations, and cancer (Peres et al. 2003). The hypothesis that exposure to toxic substances may be affecting health parameters evaluated in tapirs in the CE is an important factor that will be further evaluated in a subsequent publication.

Our data expand knowledge of the epidemiology of lowland tapirs and provide unique information on the impact of environmental health on wildlife. Increased exposure to domestic and feral animals, environmental degradation, and biodiversity loss have all been associated with increased pathogen exposure and disease risk (Pavlovsky 1966; Daszak et al. 2000; Daszak and Cunningham 2002; Epstein 2002; Cunningham et al. 2017). Our study suggests that the exposure to environmental disturbances may represent an important factor in determining the health of wild tapir populations. In addition, it suggests the possibility of using lowland tapirs as sentinel species to indicate potential health risks for other wildlife, livestock, and humans (Rabinowitz et al. 2005). We expect that results from this study might help identify threatening factors that might be overlooked in most conservation studies.

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