UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

PEDRO ENRIQUE NAVAS-SUÁREZ

Assessment of health and trauma by vehicular collisions in wild mammals from three biomes in Brazil: anthropogenic impacts on Brazilian biodiversity

São Paulo
2022

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Assessment of health and trauma by vehicular collisions in wild mammals from three biomes in Brazil: anthropogenic impacts on Brazilian biodiversity

Thesis submitted to the postgraduate program in Experimental and Comparative Pathology of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences.

Department:

Pathology

Area:

Comparative and experimental pathology

Advisor:

Prof. José Luiz Catão-Dias, Ph.D.

São Paulo

2022

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO

Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia
Universidade de São Paulo

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DO ESTADO SANITÁRIO E O TRAUMA EM MAMÍFEROS SELVAGENS ATROPELADOS EM RODOVIAS DE TRÊS BIOMAS DO BRASIL: IMPACTOS ANTRÓPICOS NA BIODIVERSIDADE BRASILEIRA", protocolada sob o CEUA nº 7198020317 (ID 005365), sob a responsabilidade de **José Luiz Catão Dias** *e equipe; Pedro Enrique Navas-Suárez* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 15/08/2018.

We certify that the proposal "EVALUATION OF HEALTH STATUS AND TRAUMA IN ROADKILL WILD MAMMALS OF THREE BRAZILIAN BIOMAS: ANTHROPIC IMPACTS IN BRAZIL BIODIVERSITY", utilizing 100 Brazilian wild species (males and females), protocol number CEUA 7198020317 (ID 005365), under the responsibility of **José Luiz Catão Dias** *and team; Pedro Enrique Navas-Suárez* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 08/15/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 04/2017 a 06/2021   Área: Patologia Experimental E Comparada

Origem:	Animais provenientes de outros projetos						
Espécie:	Espécies silvestres brasileiras		sexo: Machos e Fêmeas	idade:	00 a 00 dias	N:	100
Linhagem:	---			Peso:	00 a 00 g		

Registro IBAMA/Sisbio/Etc: Licença do IBAMA N° 215/2013 SISBIO 58745

Método de Captura: Animais mortos por atropelamento

Local do experimento: Os xenarthra serao necropsiados pelo equipe do projeto, os animais do museu serão necropsiados pelo doutorando no VPT

São Paulo, 08 de fevereiro de 2022

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Av. Prof. Dr. Orlando Marques de Paiva, 87, Cidade Universitária: Armando de Salles Oliveira CEP 05508-270 São Paulo/SP - Brasil - tel: 55 (11) 3091-7676
Horário de atendimento: 2ª a 5ª das 7h30 às 16h : e-mail: ceuavet@usp.br
CEUA N 7198020317

Ministério do Meio Ambiente - MMA

Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio

Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 58745-4	Data da Emissão: 18/01/2019 14:39:18	Data da Revalidação*: 18/01/2020

De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.

Dados do titular

Nome: Pedro Enrique Navas-Suárez	CPF: 237.789.788-61
Nome da Instituição: Faculdade de Medicina Veterinária e Zootecnia USP	CNPJ: 63.025.530/0019-33

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	coleta de carcaças nas rodovias	05/2017	12/2020

Equipe

#	Nome	Função	CPF	Nacionalidade
1	FERNANDA ABRA	Colaboradora	329.411.568-79	Brasileira

Observações e ressalvas

1	A autorização não eximirá o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VI) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.
3	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, a difusão ou a pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
4	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
5	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
6	Este documento somente poderá ser utilizado para os fins previstos na Instrução Normativa ICMBio n° 03/2014 ou na Instrução Normativa ICMBio n° 10/2010, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
7	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infra-estrutura da unidade.
8	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.

Este documento foi expedido com base na Instrução Normativa nÂº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0587450420190118

Autorização para atividades com finalidade científica

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Dados do titular

Nome: Pedro Enrique Navas-Suárez	CPF: 237.789.788-61
Nome da Instituição: Faculdade de Medicina Veterinária e Zootecnia USP	CNPJ: 63.025.530/0019-33

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Rodovias	Nova Andradina-MS	Cerrado	Não	Fora de UC Federal
2	Rodovias	Ribas do Rio Pardo-MS	Cerrado	Não	Fora de UC Federal
3	Rodovias	Nova Alvorada do Sul-MS	Cerrado	Não	Fora de UC Federal
4	Rodovias	Miranda-MS	Cerrado	Não	Fora de UC Federal
5	Rodovias	Três Lagoas-MS	Cerrado	Não	Fora de UC Federal
6	Rodovia BR116	Jacupiranga-SP	Mata Atlântica	Não	Fora de UC Federal
7	Rodovias	Santa Rita do Pardo-MS	Cerrado	Não	Fora de UC Federal
8	Rodovia SP-99	Paraibuna-SP	Mata Atlântica	Não	Fora de UC Federal
9	Rodovia BR116	Itapecerica da Serra-SP	Mata Atlântica	Não	Fora de UC Federal
10	Rodovias	Corumbá-MS	Pantanal	Não	Fora de UC Federal
11	Rodovia BR116	Miracatu-SP	Mata Atlântica	Não	Fora de UC Federal
12	Rodovia BR116	Juquitiba-SP	Mata Atlântica	Não	Fora de UC Federal
13	Rodovia BR262	Aquidauana-MS	Cerrado	Não	Fora de UC Federal
14	Rodovias	Bataguassu-MS	Cerrado	Não	Fora de UC Federal
15	Rodovia SP-99	Caraguatatuba-SP	Mata Atlântica	Não	Fora de UC Federal
16	Rodovia SP-99	São José dos Campos-SP	Mata Atlântica	Não	Fora de UC Federal
17	Rodovia BR116	São Lourenço da Serra-SP	Mata Atlântica	Não	Fora de UC Federal
18	Rodovias	Água Clara-MS	Cerrado	Não	Fora de UC Federal
19	Estradas do estado de sao paulo	SP	Cerrado	Não	Fora de UC Federal
20	Estradas do estado de Sao Paulo	SP	Mata Atlântica	Não	Fora de UC Federal
21	Rodovia MS-40	Campo Grande-MS	Pantanal	Não	Fora de UC Federal
22	Rodovias SP-99, BR-116, SP-270, BR-50, SP-348,	São Paulo-SP	Mata Atlântica	Não	Fora de UC Federal

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Coleta/transporte de amostras biológicas ex situ	Cervidae	-
2	Coleta/transporte de amostras biológicas in situ	Cervidae	-
3	Coleta/transporte de amostras biológicas ex situ	Callithrichidae	-
4	Coleta/transporte de amostras biológicas in situ	Callithrichidae	-
5	Coleta/transporte de amostras biológicas ex situ	Cingulata	-
6	Coleta/transporte de amostras biológicas in situ	Cingulata	-

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Atividades X Táxons

#	Atividade	Táxon	Qtde.
7	Coleta/transporte de amostras biológicas in situ	Aves	-
8	Coleta/transporte de amostras biológicas ex situ	Aves	-
9	Coleta/transporte de amostras biológicas in situ	Tayassuidae	-
10	Coleta/transporte de amostras biológicas ex situ	Tayassuidae	-
11	Coleta/transporte de amostras biológicas in situ	Canidae	-
12	Coleta/transporte de amostras biológicas ex situ	Canidae	-
13	Coleta/transporte de amostras biológicas ex situ	Felidae	-
14	Coleta/transporte de amostras biológicas in situ	Felidae	-
15	Coleta/transporte de amostras biológicas in situ	Mustelidae	-
16	Coleta/transporte de amostras biológicas ex situ	Mustelidae	-
17	Coleta/transporte de amostras biológicas ex situ	Procyonidae	-
18	Coleta/transporte de amostras biológicas in situ	Procyonidae	-
19	Coleta/transporte de amostras biológicas ex situ	Leporidae	-
20	Coleta/transporte de amostras biológicas in situ	Leporidae	-
21	Coleta/transporte de amostras biológicas ex situ	Tapiridae	-
22	Coleta/transporte de amostras biológicas in situ	Tapiridae	-
23	Coleta/transporte de amostras biológicas in situ	Cebidae	-
24	Coleta/transporte de amostras biológicas ex situ	Cebidae	-
25	Coleta/transporte de amostras biológicas in situ	Atelidae	-
26	Coleta/transporte de amostras biológicas ex situ	Atelidae	-
27	Coleta/transporte de amostras biológicas in situ	Agoutidae	-
28	Coleta/transporte de amostras biológicas ex situ	Agoutidae	-
29	Coleta/transporte de amostras biológicas in situ	Dasyproctidae	-
30	Coleta/transporte de amostras biológicas ex situ	Dasyproctidae	-
31	Coleta/transporte de amostras biológicas in situ	Erethizontidae	-
32	Coleta/transporte de amostras biológicas ex situ	Erethizontidae	-
33	Coleta/transporte de amostras biológicas in situ	Muridae	-
34	Coleta/transporte de amostras biológicas ex situ	Muridae	-
35	Coleta/transporte de amostras biológicas in situ	Caviidae	-
36	Coleta/transporte de amostras biológicas ex situ	Caviidae	-
37	Coleta/transporte de amostras biológicas in situ	Bradypodidae	-
38	Coleta/transporte de amostras biológicas ex situ	Bradypodidae	-
39	Coleta/transporte de amostras biológicas in situ	Myrmecophagidae	-
40	Coleta/transporte de amostras biológicas ex situ	Myrmecophagidae	-
41	Coleta/transporte de amostras biológicas in situ	Didelphidae	-

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Atividades X Táxons

#	Atividade	Táxon	Qtde.
42	Coleta/transporte de amostras biológicas ex situ	Didelphidae	-
43	Coleta/transporte de amostras biológicas ex situ	Mephitidae	-
44	Coleta/transporte de amostras biológicas in situ	Mephitidae	-
45	Coleta/transporte de amostras biológicas in situ	Didelphidae	-
46	Coleta/transporte de amostras biológicas ex situ	Didelphidae	-

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Aves)	Ectoparasita, Fezes, Animal encontrado morto ou partes (carcaça)/osso/pele, Fragmento de tecido/órgão
2	Amostras biológicas (Carnívoros)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fezes, Fragmento de tecido/órgão, Pêlo, Regurgitação/conteúdo estomacal, Sangue
3	Amostras biológicas (Outros mamíferos)	Animal encontrado morto ou partes (carcaça)/osso/pele, Fragmento de tecido/órgão, Fezes, Ectoparasita, Pêlo, Regurgitação/conteúdo estomacal, Sangue
4	Amostras biológicas (Primatas)	Animal encontrado morto ou partes (carcaça)/osso/pele, Fragmento de tecido/órgão, Ectoparasita, Fezes, Pêlo, Regurgitação/conteúdo estomacal, Sangue
5	Amostras biológicas (Tamanduás)	Animal encontrado morto ou partes (carcaça)/osso/pele, Fezes, Ectoparasita, Fragmento de tecido/órgão, Pêlo, Regurgitação/conteúdo estomacal, Sangue
6	Amostras biológicas (Tatus)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Fezes, Pêlo, Regurgitação/conteúdo estomacal, Sangue
7	Método de captura/coleta (Carnívoros)	Outros métodos de captura/coleta(Carcacas atropeladas nas rodovias)
8	Método de captura/coleta (Outros mamíferos)	Outros métodos de captura/coleta(Carcacas atropeladas nas rodovias)
9	Método de captura/coleta (Tamanduás)	Outros métodos de captura/coleta(Carcacas atropeladas nas rodovias)

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Página 4/6

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Nome da Instituição: Faculdade de Medicina Veterinária e Zootecnia USP	CNPJ: 63.025.530/0019-33

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	Faculdade de Medicina Veterinária e Zootecnia USP	Outro

Autorização para atividades com finalidade científica

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Nome da Instituição: Faculdade de Medicina Veterinária e Zootecnia USP	CNPJ: 63.025.530/0019-33

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

Código de autenticação: 0587450420190118

EVALUATION FORM

Autor: NAVAS-SUÁREZ, Pedro Enrique

Title: **Assessment of health and trauma by vehicular collisions in wild mammals from three biomes in Brazil:** anthropogenic impacts on Brazilian biodiversity

Thesis submitted to the postgraduate program in Experimental and Comparative Pathology of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences.

Date: _____/_____/_____

Committee members

Prof. _____

Institution: _____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

This thesis is dedicated to the wildlife that, giving their lives, allowed me to carry out this research.

Esta tesis es dedicada a cada uno de los animales silvestres que dando su vida me permitieron desarrollar esta investigación.

ACKNOWLEDGEMENTS

Since I began my training process as a researcher (first in my master's degree and now in my doctorate), I have always been aware of how fortunate I was, since the number of inhabitants who complete a doctorate is small, only in Colombia, the rate of citizens with a Ph.D. is 16 per million inhabitants. One day reading literature, I came across my friend Fernanda Abra's thesis in which she expressed, "The Ph.D. goes beyond a title, it is a mark in the life on "***noblesse oblige***"... "noblesse oblige" or "noble obligation" is a french expression and mean that with a title, prestige or anything that brings you prominence in society also comes the responsibility." Those paragraphs had a powerful effect on me. For this reason, at this moment of finishing my doctoral training, I am convinced that the seven years of my life that I have dedicated to this process have given me enough tools to fulfill my "noblesse oblige" from now on. I am very grateful to life and to god for allowing me to meet the right people at the correct times. From now on, the other paragraphs of gratitude will be written in the native languages of those to whom I will be eternally grateful.

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Talvez este seja o parágrafo mais difícil de escrever, pois muitos sentimentos vêm à mente, muitos momentos compartilhados, conversas sobre diversos temas, professor Catão, sempre me impressionou sua postura, o respeito que o senhor gera, a quantidade e qualidade de conhecimento que o senhor tem, mas sobretudo a sua simplicidade e proficiência em se comunicar com seus alunos e orientá-los. O senhor é meu maior exemplo de ser humano. Lembro-me perfeitamente daquele dia de agosto de 2012, no qual Isabela Furegatti bateu em sua porta para apresentar seu mais novo intercambista, eu confesso que estava bastante nervoso porque ia conhecer um dos editores do livro de medicina de vida selvagem mais importante na América Latina, naquele dia você falou comigo em espanhol e me apresentou individualmente aos seus alunos, aos professores e funcionários do departamento. Naquela época eu estava bastante confuso, e não entendia como uma pessoa tão importante e ocupada estava tomando o tempo para me apresentar individualmente, naquele dia despertou sentimento de pertencimento e de que estava no local certo

(esse ensino levarei pelo resto da minha vida). Professor, agradeço cada momento que você me dedicou e sempre estarei grato ao senhor, levando com a maior responsabilidade meu conhecimento para executar minha "noblesse oblige" com a proficiência que o senhor ó faz. Seja o seja que a vida me leve sempre serei um lapconiano motivado em aprender e ensinar, obrigado.

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Realizar un doctorado no es algo sencillo, son muchas horas de dedicación que a veces implican en perder un poco el control de la vida, son horarios extra de trabajo y noches completas estudiando. Esto resulta en muchos sacrificios, en mi caso estar lejos de mi familia fue muy difícil, no obstante, durante el primer perioro del doctorado sabia que todos los días conversaba con esa persona que me daba equilibrio y era muy satisfactorio, fueron casi dos años a distancia y actualmente conseguimos crear nuestro hogar. Durante este doctorado a parte de tener un titulo, pude consolidar la felicidad de tener una gran persona en mi vida, mi amor, mi amiga, mi apoyo, mi mayor fan, mi consultora, MI ESPOSA, Catalina tu me haces ser mejor persona, me haces sentirme amado y me haces saber que todas las cosas que viva en mi vida sean

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"Always dream and shoot higher than you know you can do. Do not bother just to be better than your contemporaries or predecessors. Try to be better than yourself".

— William Faulkner

"The researcher suffers the disappointments, the long months spent in the wrong direction, the failures. But failures are also useful, because, well analyzed, they can lead to success. And for the researcher, there is no joy comparable to that of a discovery, no matter how small…"

— Sir Alexander Fleming

"Discovery consists of seeing what everybody has seen and thinking what nobody has thought".

— Albert Szent-Györgyi

"It is not the strongest of the species that survives, nor the most intelligent; it is the one most adaptable to change".

— Charles Darwin

RESUMO

NAVAS-SUÁREZ, P. E. **Avaliação do estado sanitário e do trauma em mamíferos selvagens atropelados em rodovias de três biomas do Brasil:** impactos antrópicos na biodiversidade brasileira**.** 2022. 276 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2022.

A mortalidade direta por atropelamento é uma das principais ameaças à vida selvagem relacionada às estradas. No Brasil, a mortalidade anual estimada de animais selvagens nas estradas varia de 14 a 475 milhões de indivíduos. Além disso, o Banco Mundial estima que pelo menos 25 milhões de quilômetros de novas rodovias serão construídas nos próximos 30 anos, principalmente em países em desenvolvimento de regiões tropicais. Assim, espera-se que a mortalidade por atropelamento seja uma séria ameaça à biodiversidade se as estradas não se adequam ambientalmente (por exemplo, cercas, passagens inferiores, viadutos). Simultaneamente, considerando que alguns aspectos sanitários da biodiversidade neotropicais ainda precisam ser esclarecidos; o atropelamento apresenta-se como uma excelente fonte oportunista de dados/amostras. Nesse contexto, esta tese de doutorado teve como objetivo ampliar o conhecimento sobre as lesões traumáticas do atropelamento em mamíferos selvagens coletados em três biomas brasileiros; além disso, como contribuição ao conhecimento da saúde dos mamíferos selvagens neotropicais, foram realizados estudos patológicos, moleculares, parasitológicos e toxicológicos. Em síntese, no capítulo 1 é apresentada uma revisão da literatura, que busca contextualizar o desenvolvimento rodoviário no Brasil desde as perspectivas políticas, econômicas e ambientais. A seguir, o capítulo 2 apresenta uma descrição dos padrões topográficos do traumatismo por atropelamento nas diversas espécies estudadas. Por fim, o capítulo 3 é os apêndices detalham os aspectos da saúde. A revisão de literatura sugere que interesses políticos e econômicos foram importantes para que o governo brasileiro optasse pelo desenvolvimento rodoviário em detrimento de outros modais de transporte, como ferrovias e hidrovias. Embora o Brasil tenha uma das redes rodoviárias mais extensas do mundo, a falta de orçamento tornou difícil a manutenção da rede, o que explica a baixa qualidade e a mínima adequação ambiental de algumas estradas. Além disso, a legislação ambiental é relativamente

nova, iniciando sua aplicação na década de 80. Atualmente, diversas rodovias (concessionadas e federais/estaduais) reconhecem seus impactos ambientais e implementaram programas de mitigação e compensação. No entanto, ainda existem projetos rodoviários que podem ser prejudiciais à biodiversidade. Subsequentemente, a compressão biomecânica das lesões por atropelamento ajuda a entender melhor sua distribuição e gravidade. Assim, este autor considera que a descrição e frequência das lesões para cada um dos táxons estudados pode auxiliar os veterinários que realizam o atendimento emergencial de espécimes que permanecem vivos após o traumatismo. Por fim, dentre os principais resultados sobre os aspectos sanitários destacasse a descrição da adiaspiromicose pulmonar, doença fúngica zoonótica negligenciada em duas espécies de tatus, assim como um estudo toxicológico detectando três pesticidas atualmente banidos em algumas espécies de carnívoros selvagens. Este autor acredita firmemente que os resultados aqui apresentados podem ser de grande importância para sensibilizar à sociedade sobre os impactos negativos das estradas sobre a biodiversidade; da mesma forma, do ponto de vista de uma saúde, o uso desses espécimes em programas nacionais ou estaduais de vigilância sanitária pode ser uma notável estratégia.

Palavras-chave: Trauma contuso. Conservação. Patologia Veterinária. Saúde da Vida Selvagem. Atropelamento.

ABSTRACT

NAVAS-SUÁREZ, P. E. **Assessment of health and trauma by vehicular collisions in wild mammals from three biomes in Brazil:** anthropogenic impacts on Brazilian biodiversity. 2022. 276 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2022.

Direct mortality from motor vehicle collisions (MVC) is one of the greatest road-related threats to wildlife. In Brazil, the estimated annual wildlife mortality on the road ranges from 14 to 475 million individuals. Furthermore, the World Bank estimates that at least 25 million kilometers of new roads will be built in the next 30 years, mainly in developing countries in tropical regions. Thus, road mortality is expected to be a serious threat to biodiversity if planned and built roads do not implement an environmental program (e.g., fences, underpasses, overpasses). Simultaneously, considering that some health aspects of Neotropical biodiversity still need to be elucidated, MVC is presented as an excellent opportunistic source of data/samples. In this context, this doctoral thesis aimed to expand the knowledge about the MVC-traumatic injuries (MVC-TI) in wild mammals collected in three Brazilian biomes; in addition, as a contribution to the health data of neotropical wild mammals, pathological, molecular, parasitological, and toxicological studies were carried out. Chapter 1 presents a literature review, which seeks to contextualize road development in Brazil from political, economic, and environmental perspectives. Chapter 2 presents a detailed description of the topographical patterns of MVC-TI in the different species of wild mammals studied. Finally, chapter 3 and appendix detail health aspects. The literature review suggests that political and economic interests were important for the Brazilian government to opt for road development over other transport modes, such as railroads and waterways. Although Brazil has one of the most extensive road networks globally, the lack of budget makes it challenging to maintain the network, which explains some roads' low quality and minimal environmental suitability. Furthermore, environmental legislation is relatively new, beginning in the 1980s. Some highways (toll and federal/state) currently recognize their environmental impacts and have implemented mitigation and compensation programs. However, there are still road projects that can be harmful to biodiversity. Subsequently, the biomechanical

compression of MVC-TI helps understand the distribution and severity of these injuries. So, this author considers that the description and frequency of the lesions observed for each taxon studied can help veterinarians who perform emergency care for specimens that remain alive after MVC. Finally, among the main results on health aspects, it is highlighted the description of pulmonary adiaspiromycosis, a neglected zoonotic fungal disease in two armadillo species, and a toxicology study detecting three currently banned pesticides in some wild carnivore species. This author confidently believes that the results presented here can be of great importance to make society aware of the negative impacts of roads on fauna; likewise, from a one health perspective, the use of these specimens in national or state health surveillance programs seems to be a remarkable strategy.

RESUMEN

NAVAS-SUÁREZ, P. E. **Evaluación del estado sanitario y del trauma por atropello en mamíferos silvestres de tres biomas de Brasil:** impactos antrópicos em la biodiversidad brasilera. 2022. 276 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2022.

La mortalidad directa por atropello es una de las principales amenazas para la vida silvestre relacionadas con las carreteras. En Brasil, la mortalidad anual estimada de animales silvestres en carreteras varía de 14 a 475 millones de individuos. Además, el Banco Mundial estima que serán construidos al menos 25 millones de kilómetros de nuevas carreteras en los próximos 30 años, principalmente en países en desarrollo de regiones tropicales. Por lo tanto, se espera que la mortalidad por atropello sea una amenaza grave para la biodiversidad si las carreteras no son ambientalmente adecuadas (p. ej., vallas, pasos subterráneos, viaductos). Simultáneamente, considerando que algunos aspectos sanitarios de la biodiversidad Neotropical aún deben ser esclarecidos; el atropello se presenta como una excelente fuente oportunista de datos/muestras. En este contexto, esta tesis doctoral tuvo como objetivo ampliar el conocimiento sobre las lesiones traumáticas causadas por atropellos en mamíferos silvestres recolectados en tres biomas brasileños; además, como aporte al conocimiento de la salud de los mamíferos silvestres neotropicales, se realizaron estudios patológicos, moleculares, parasitológicos y toxicológicos. En resumen, el capítulo 1 presenta una revisión de la literatura, que busca contextualizar el desarrollo vial en Brasil desde las perspectivas política, económica y ambiental. A continuación, el capítulo 2 presenta una descripción de los patrones topográficos de las lesiones por atropello en las diferentes especies estudiadas. Finalmente, el capítulo 3 y los apéndices detallan los aspectos sanitarios. La revisión de la literatura sugiere que intereses políticos y económicos fueron importantes para que el gobierno brasileño optara por el desarrollo de carreteras sobre otros modos de transporte, como ferrocarriles y vías fluviales. Aunque Brasil tiene una de las redes viales más extensas del mundo, la falta de presupuesto ha dificultado el mantenimiento de la red, lo que explica la baja calidad y la mínima adecuación ambiental de algunas carreteras. Además, la legislación ambiental es relativamente nueva, comenzando a aplicarse

solo en la década de 1980. Actualmente, varias carreteras (concesionadas y federales/estatales) reconocen sus impactos ambientales y han implementado programas de mitigación y compensación. Sin embargo, aún existen algunos proyectos viales que podrían ser perjudiciales para la biodiversidad. Posteriormente, la compresión biomecánica de las lesiones por atropello ayuda a comprender mejor su distribución y gravedad. Así, este autor considera que la descripción y frecuencia de lesiones para cada uno de los taxones estudiados puede ayudar a los médicos veterinarios que brindan atención de emergencia a los ejemplares que quedan vivos después del atropello. Finalmente, entre los principales resultados de los aspectos de salud, son destacadas la descripción de la adiaspiromicosis pulmonar, una enfermedad fúngica zoonótica desatendida en dos especies de armadillos, así como, un estudio de toxicología que detectó tres pesticidas actualmente prohibidos en algunas especies de carnívoros salvajes. Este autor cree con confianza que los resultados aquí presentados pueden ser de gran importancia para sensibilizar a la sociedad sobre los impactos negativos de las carreteras sobre la biodiversidad; asimismo, desde el punto de vista de una salud, el uso de estos especímenes en programas de vigilancia sanitaria nacionales o estatales puede ser una estrategia destacable.

SUMMARY

1 INTRODUCTION

Land transportation is fundamental to modern societies. Several studies have shown that the quality of a country's road network influences its inhabitants' economic and social development (BRÖCKER & RIETVELD, 2009; AMADOR-JIMENEZ & WILLIS, 2012; WEISS *et al.*, 2018). According to the Central Intelligence Agency (CIA) of the United States of America, in 2013, the total kilometers (km) of roads on the planet exceeded 64.2 million (CIA, 2021). Additionally, by 2050 it is estimated that 25 million kilometers of new roads will be built, mainly in tropical and subtropical regions (LAURANCE *et al.*, 2014). In sharp contrast, roads represent one of the greatest threats to biodiversity worldwide (FORMAN & ALEXANDER, 1998; TROMBULAK & FRISSELL, 2000). For wildlife, deleterious effects include the loss or reduction of habitat quality (e.g., noise, artificial lighting, pollution, and visual disturbance) (FORMAN *et al.*, 2003; EIGENBROD *et al.*, 2009; PARRIS *et al.*, 2009), the barrier effect (e.g., interruption of migration or dispersion) (NELLEMANN *et al.*, 2001; VISTNES *et al.*, 2004; LESBARRERES & FAHRIG, 2012), and direct mortality by motor-vehicle collisions (MVC) (FORMAN & ALEXANDER, 1998; FAHRIG & RYTWINSKI, 2009). In addition, road mortality can modify the demographic structure of wildlife populations, with the chance of leading to local sinks (STEEN & GIBBS, 2004; NIELSEN *et al.*, 2006). It is noteworthy that the severity of road impacts depends on road density, traffic volume, landscape structure, proximity to protected areas, and wildlife diversity (AMENT *et al.*, 2008; FRAIR *et al.*, 2008; DE FREITAS *et al.*, 2015; RYTWINSKI & FAHRIG, 2013).

The Brazilian scenario is fascinating since it has the fourth-largest road network in the world, with just over 2 million km of roads (CIA, 2021) and shelters between 15 and 20% of the planet's total biodiversity (ICMBIO, 2020). In addition, two main factors influence the impact of roads on Brazilian biodiversity. First, available data indicate that between 14.7 (±44.8) million and 474 million animals die by MVC on Brazilian roads per year (CBEE, 2021; DORNAS *et al.*, 2012). The excessive number of fatalities per year on Brazilian roads may provide a unique opportunity to gather information about these species' health aspects (e.g., presence of infectious agents, exposure to pollutants, parasitic diversity) that would be otherwise difficult to obtain. Second, from

the perspective of integrated health (or "One health"), knowledge of ecosystem health provides a better understanding of issues, and it may help prevent diseases that affect both humans and animals (domestic, production, and wild), as well as the environment (STÄRK *et al.*, 2015). Furthermore, considerable emerging and re-emerging human pathogens can originate in wildlife. Therefore, health assessment, surveillance, and wildlife monitoring are highly relevant and should be part of the national disease surveillance programs (DASZAK *et al.*, 2000; MORNER *et al.*, 2002). In Brazil, few studies have surveyed pathogens of human relevance in carcasses of roadkilled wild animals (RICHINI-PEREIRA *et al.,* 2008-2014; SPOLIDORIO *et al.* 2012); therefore, wildlife does not yet play a key role in national infectious disease surveillance programs.

In this context, this doctoral thesis aimed to assess the health and characteristics of MVC injuries in wild mammals in three Brazilian biomes, namely Mata Atlântica, Pantanal, and Cerrado. For this, **Chapter 1** show a literature review on the history of roads in Brazil, providing an economic, political, and environmental contextualization. In addition, the anatomopathological characterization of injuries caused by vehicular collisions is explained in **Chapter 2** and the health status in **Chapter 3**. As a result, a 36-month longitudinal study was formulated and carried out. Besides, this study made it possible to collect corpses of wild mammals from roads in four Brazilian states (São Paulo, Santa Catarina, Paraná, and Mato Grosso do Sul) through partner institutions.

Briefly, **Chapter** 1 aims to present a concise historical description of Brazil's land transport and road development. As mentioned in the previous paragraphs, Brazil has an extensive network of highways distributed throughout the entire national territory. The roads in Brazil date from the colonial period, although indeed, before the colonial period, the native communities already had their paths. Until the 20s of the 20th century, Brazilian terrestrial transport was almost entirely by trains, and even the train network interconnected the main cities of the Atlantic coast. The railway development was largely due to the transport of commodities such as coffee. Between the 30s and 60s, road construction was significant, and Brazil adopted a road modal to the detriment of trains. By the 80s, the country already had more than 47 thousand km of paved roads. It is noteworthy that concern for the environmental impacts

associated with the construction and use of highways began in the 1980s with the approval of the national environmental policy. In this way, the environmental licensing began, seeking mitigation and compensation of environmental impacts. Thanks to advances in the national environmental legal framework, several road projects consider environmental impacts and create forms of mitigation and compensation. Therefore, several highways have mitigation structures to reduce the effects of collisions, such as fences and wildlife crossings. However, many highway projects still put biodiversity at risk; a good example is highway BR-319, which can increase deforestation of the Amazonian arc, and the continuation of highway BR-364 that connects the state of Acre with Peru. To summarize, highways have played a fundamental role in developing land transport in Brazil. On the other hand, environmental regulations are relatively recent and are still not applied to all roads today.

Chapter 2 compiles two scientific articles to describe the information corresponding to the anatomopathological study of injuries caused by vehicular collisions in the investigated animals. The first shows a pilot study carried out in Lowland Tapirs (*Tapirus terrestris*). It was used species as a pilot, once at the beginning of this research, we already had enough information to analyze. Based on the autopsy reports, we applied the concepts of biomechanics and trauma to describe and categorize the findings in the necropsy. The second one compiles the data of all the species examined. To summarize, we consider this the first large-scale study to evaluate the injuries observed in wild mammals' victims of vehicular collisions in Brazil. It was possible to identify injuries from blunt trauma, as well as from a perforated pattern. Furthermore, we verify that diagnostic images (X-rays and tomography) play a fundamental role in the post-mortem analysis of this process. Additionally, we seek to determine the association of biological characteristics in the observed trauma patterns.

Finally, **Chapter 3** details the health aspects of the wild mammal cohort included in this doctoral thesis. Two postulates were fundamental for the construction of this chapter; Firstly, an animal with a health disorder, whether infectious or non-infectious (e.g., a neuropathy), must be more susceptible to a vehicular collision. Secondly, wildlife harbors many significant pathogens for humans and animals (e.g., rabies virus,

Hansen's disease, *Toxoplasma gondii*). Therefore, this chapter presents a compilation of scientific articles from the research seeking to describe, analyze and discuss the non-traumatic processes observed in these animals. For a better reading, we compiled the studies as follows:

- Morphological and molecular characterization of a neglected fungal agent (adiaspiromycosis in armadillos);
- Presence of pesticides in carnivores;
- The diversity of ectoparasites and endoparasites (appendix).

Through the contextualization of road development in Brazil, it is evident that the problem of road impacts on biodiversity is historical, and the last three chapters demonstrate the size and importance of road mortality in Brazil. However, this author considers that despite being recent, the Brazilian legal framework presents regulations that seek to mitigate and compensate for the environmental effects of highways. Indeed, this author believes that based on the information obtained in this doctoral thesis, the road mortality of wild mammals is an anthropic process that affects a great diversity of species in the three biomes studied. Additionally, the animals presented good health characteristics, which leads to thinking of potentially suitable animals for their reproduction

The results here are presented to emphasize that cadavers originating from MVC are essential material for monitoring infectious pathogens and dangerous substances such as pesticides. This author hopes that the results could be helpful to the scientific community, decision-makers, social leaders, and the general population. We know that biodiversity is part of our culture, and we must strive to preserve it.

2   CHAPTER I: ROADS AND BIODIVERSITY FROM THE COLONY TO THE 21ST CENTURY: HISTORY, DEVELOPMENT, IMPACTS AND PERSPECTIVES IN BRAZIL
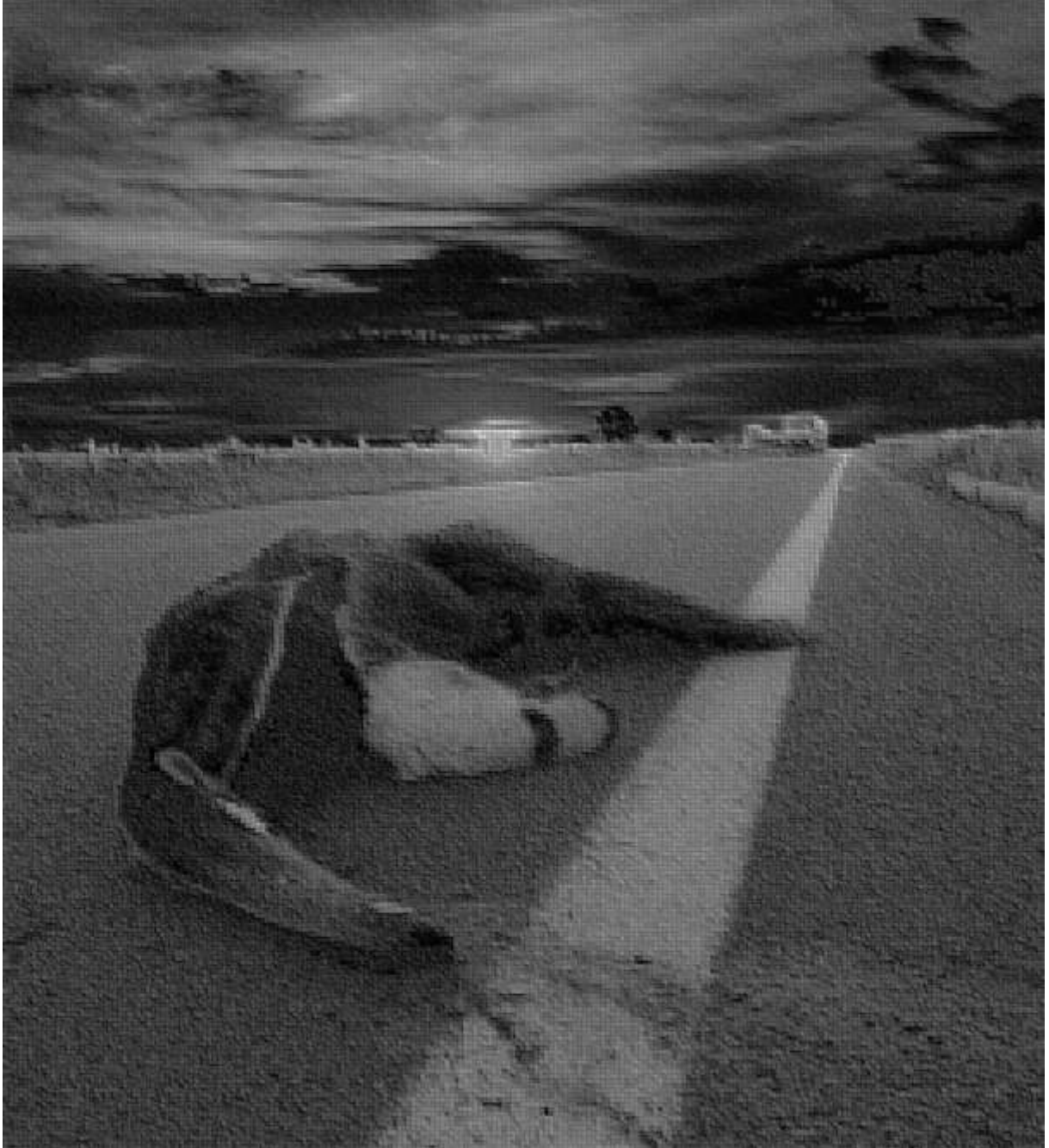
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Chapter I: Roads and Biodiversity from the Colony to the 21st Century: History, Development, Impacts and Perspectives in Brazil

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2.1   Abstract

In Brazil, terrestrial transport historically was strongly associated with road development. The objective of this manuscript is to present a historical contextualization of Brazilian road development. Additionally, a literature review offers the research data on road mortality of biodiversity in Brazil. Finally, we seek to present, discuss, and reflect on the future of highways and the effectiveness of mitigation strategies used in the country's road network. Political decisions led the government to opt for a road modality to the detriment of railways and waterways. As a result, Brazil has the fourth-largest road network on the planet (+/- 1.7 million km). Nonetheless, only 12.4% corresponds to paved roads. Additionally, in the last 20 years, the network grew just 0.5%, the roads deteriorate more every day, and there are no clear changes in the federal budget to improve its quality. Furthermore, research has shown that roads cause negative impacts on the environment. One of the significant impacts on wildlife is the increase in unnatural mortality by Motor Vehicle Collisions (MVC). In Brazil, this is a well-documented impact affecting almost all vertebrate species throughout the national territory. In a literature review, from 1988 to 2021, we detected 92 studies (articles, technical reports, congress summaries, theses, and dissertations) where authors report the mortality of 108,970 vertebrates on roads in 24 of the 27

federative units. In the Brazilian legal framework, several laws, decrees, and regulations regulate the mitigation of the environmental impacts of highways. In addition, these regulations are responsible for stipulating the requirements for environmental licenses. However, these regulations only began in the early 1980s. Currently, several highways have mitigation structures to reduce the roadkill impact, such as fences and wildlife passages, but many current highway projects still put biodiversity at risk. Therefore, road ecology is an area that has excellent development potential in the country.

Keywords: Conservation; defaunation; road ecology; wildlife.

2.2   Introduction

Terrestrial transportation has been one of the essential development pillars of human society (AMADOR-JIMENEZ & WILLIS, 2012). Our ancestors needed to transport themselves initially on foot, later using animals, and then building automotive vehicles. In the 21st century, the challenge will be to create efficient autonomous vehicles with renewable energy sources (HERBST, 2005; HUSSAIN & ZEADALLY, 2018). However, it is noteworthy that road construction in any part of the world will always be to the detriment of the environment since the construction of roads implies deforestation (NOGUEIRA & NETO, 2016; MEIJER *et al.*, 2018). Additionally, roads and traffic can favor the dispersal of exotic species (flora and fauna), affect the movement patterns (e.g., migration, isolation), reduce populations of wild animals (by an increase of unnatural mortality), and causes economic losses (compensations, medical and garage costs) (FORMAN & ALEXANDER, 1998; FORMAN *et al.*, 2003; FAHRIG & RYTWINSKI, 2009).

Brazil has a territorial area that exceeds 8.5 million km^2 (fifth in the world) and inhabits more than 211 million people (sixth most populated) (CIA, 2021). Its road development is noteworthy since it passed from 423 km of paved roads (in the 20th century 40s) to more than 47 thousand km in the 80s; Currently, the road network

exceeds 1.7 million km, of which 221,820 km correspond to paved roads (CIA, 2021). Brazil has the fourth most extensive road network globally and is a megadiverse country, for which the impacts of roads on biodiversity could be expressive. This chapter aims to present a historical contextualization of Brazilian road development and its relationship with the preservation of biodiversity. In addition, we intend to understand research trends on the impacts of roads on biodiversity through a literature review. Finally, we display reflections and discussions on the future of roads and the effectiveness of mitigation strategies used in the country's road network.

2.3   Roads in Brazil: past and present.

Even though the first paved highway in Brazil was inaugurated in 1861 by Dom Pedro II, the history of terrestrial transport is much older. In 1808, with the royal family's arrival in Brazil, there was massive densification of Rio de Janeiro city, passing from 15,000 to 57,000 inhabitants. This process induced the expansion of the inhabited territory. In this way, the first demand was generated for a public transport service that could transport people within the city's limits. However, until 1859, Dom Pedro II inaugurated the first public transport system, the animal-drawn tram network (MINISTÉRIO DA INFRAESTRUTURA, 2021). Between 1880 and 1930, the railway network developed hugely, leaving roads in the background. The agricultural sector drove Brazilian railway development. However, in 1917, during the first São Paulo state road congress, the Permanent Association of Roads (*Associação Permanente de Estradas de Rodagem*, in Portuguese) was installed, which was enacted by Law n° 2,437 of 1921. This is the first Brazilian legislative act that sought to establish an integrated road network at least at a regional level. The first president to play a fundamental role in road planning was Washington Luís (1926-1930); his administration motto was "to govern is to open roads" (*Governar é abrir Estradas*, in Portuguese). As a result, the National Highways Department (DNER) was conformed, and the Rio-Petrópolis Highway was paved with asphalt (RODRIGUES & LIBERATTI, 2019). Later, during the Getúlio Vargas government (1930-1945), the National Highway Plan was proposed, with which the implementation of a transportation network that would integrate the Brazilian territory through highways was projected;

however, this plan was not fully executed (BARAT, 1996; MINISTÉRIO DA INFRAESTRUTURA, 2021).

Only at the beginning of the 1940s, the road development policies in detriment of railways were made. The governors justified this policy because it was faster, cheaper, and offered a greater integration of the Brazilian territory. Through Law n° 8,463 of 1945, a legal framework was created to maintain investments in road development through the National Highway Fund. This law was known as the "Joppert Law", and it allowed almost all states to create their road departments (*Departamento of Estradas e Rodagem* - DER, in Portuguese) and thus design and prepare state road plans (SANTOS & SILVEIRA, 2001). In the early 50s, the Brazilian road development triggered the international automotive industry to create great expectations about the potential of the national market (CORREA GALVÃO, 1966). Some historians believe that this stimulus influenced political decisions on roads to the detriment of the railways. Since in the long term, it could be detrimental to the country's economy because road transport represents a higher cost per kilometer (ACCORSI, 1996; BARTHOLOMEU, 2006).

Juscelino Kubitschek (1956-1961) also had a relevant role in the country's road development. His government had as its fundamental axis the strategic plan "50 years in 5", with which he sought to improve the country's infrastructure in record time (BIELSCHOWSKY, 1996). By creating the Executive Group of the Automotive Industry (GEIA, in Portuguese), the national production of automotive vehicles was favored, and the paving of roads increased (FONSECA, 1955; FAUSTO, 2006). During the military dictatorship (1964-1985), the development of highways was maintained to communicate the country's different regions. In 1967, the National Highways Council was created to plan new highways and order the administration of pre-existing highways, forming the National Highways System. The road budget was based on increasing taxes and internal and external indebtedness (PEREIRA, 1973; NOGUEIRA & NETO, 2016). In the early 1970s, the highway network interconnected almost all country regions; however, at the end of this decade, the road network and the automotive industry suffered a tremendous global lethargy due to the oil crisis (MINISTÉRIO DA INFRAESTRUTURA, 2021).

During the 80s and 90s, due to the international crisis, there was a stagnation of the industrial production of motor vehicles and a deterioration of the roads (MINISTÉRIO DA INFRAESTRUTURA, 2021). However, at the end of the 90s, transport participation in the Brazilian Growth Domestic Product - GDP increased from 3.7% to 4.3%. In addition, data presented in 2000 showed that the transport sector grew around 400% between the 70s and 2000, demonstrating the importance of this sector in the Brazilian economy (ACCORSI, 1996; FLEURY, 2003; BARTHOLOMEU, 2006; TEDESCO *et al.*, 2011). It is important to note that the country's road development did not have any significant environmental concern during all this time. Nevertheless, in 1981, through the issuance of Law n° 6,938 of 1981, environmental licensing was stipulated through the National Environmental Policy (DNIT, 2021). Through this law, the National Environmental System (*SISNAMA*, in Portuguese) and the National Environmental Council (*CONAMA*, in Portuguese) were created to regulate the environmental licensing process. For this reason, in 1986, CONAMA resolution n° 01 was published describing the requirements, such as environmental impact studies and reports. These institutes were unified in 1989, creating the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, in Portuguese), which still operates today (IBAMA, 2021).

The National Transport Confederation (*CNT*, in Portuguese) identified that in 2007 74.9% of the country's highways had conservation problems; Similarly, Getulio Vargas Transport Foundation (Fundação Getúlio Vargas Transportes - FGVT) indicated that between 2008 and 2018, public investments in transport infrastructure were 0.31% of annual GDP, evidencing a reduced budget. The 2019 CNT bulletin indicates that the public investments in transportation infrastructure corresponded to 0.14% of the GDP, this being the lowest percentage in 12 years (COLAVITE & KONISHI, 2015). Only in 2013, through the issuance of inter-ministerial decree n° 288, was the Environmentally Sustainable Federal Highways Program (*PROFAS*, in Portuguese) resolution created. Through this program, the transportation and environment ministries aimed to improve the country's federal highways (DNIT, 2021).

In the last three decades, the number are not encouraging. Official data detail that at least 69% of the paths are regular or poorly maintained. The neglect of roads includes a lack of maintenance, recovery, and vigilance, increasing the number of

accidents and insecurity (ACCORSI, 1996). Right now, the country's economy is highly dependent on road transport, and the truck drivers ("caminhoneiros") associations become a strategic political force. Studies detail that in a context of balance in the Brazilian transport, costs of more than 2,500 million dollars per year would be avoided, reducing freight by up to 62% using waterways and 37% using railways (TEDESCO *et al.*, 2011; COLAVITE & KONISHI, 2015). Currently, the road network exceeds 1.7 million km (the fourth in the world), of which only 12.4% (221,820 km) are paved, while the rail network is made up of 29,165 km and the network 19.5 km hydro-railway, evidencing this imbalance (ANTT, 2019; CNT, 2020). Nevertheless, by implementing the PROFAS program and some state regulations, Brazilian roads are suffering essential investments to mitigate these impacts.

The history of road development in Brazil dates back to the colonial era (1820). Likewise, extensive investments were made to make Brazil a leading country in its road structure, but due to budgetary problems, the quality of the roads is currently regular. This excessive number of roads associated with weak environmental legislation that was only initiated until 1980 helps us to contextualize the results presented below on the impacts of roads on biodiversity. Supplementary table 1 shows the primary laws, regulations, and decrees related to transportation.

2.4 Impacts of roads in Brazil

Human safety & economics

In Brazil, according to data from the road accident statistics of the National Department of Transportation Infrastructure (DNIT, in Portuguese), between 2007-2011, more than 800,000 accidents were registered. In 2020, 33,530 people died in traffic accidents, detailing a higher incidence of men aged between 25 to 44 years (SOBANSKI et al., 2013; CNT, 2020; SEGURADORA LIDER, 2020). Although in the world, 1.3 million people die in this type of accident, age and gender pattern is the same as described in Brazil according to the WHO; 93% of the world's fatalities on the roads occur in low- and middle-income countries, even though these countries have approximately 60% of the world's vehicles (WHO, 2018). According to ABRA *et al.* (2019), data from the São Paulo Military Highway Police detail that between 2003 and

2013, there were 2,611 vehicle accidents with animals annually, of which 18.5% resulted in human injuries /or deaths. The total annual cost for the toll roads was estimated at R$ 56,550,642 (US $ 25,144,794). Regardless of whether there were human injuries or deaths, the average cost of a single accident was R $ 21,656 (US $ 9,629). Road administrators spent an average of R $ 2,463,380 (US $ 1,005,051) per year in compensation for victims.

Biodiversity

Roads represent one of the greatest threats to biodiversity (FORMAN & ALEXANDER, 1998; TROMBULAK & FRISSELL, 2000). For wildlife, the effect of roads and traffic ranges from loss of habitat (FORMAN, 2003), reduction of habitat quality in areas adjacent to roads (e.g., acoustic, artificial lighting and visual pollutions (EIGENBROD *et al.*, 2009; PARRIS *et al.*, 2009), barrier effect, including the interruption of migration and dispersal (NELLEMANN *et al.*, 2001; VISTNES *et al.*, 2004; LESBARRERES & FAHRIG, 2009) and direct mortality from vehicular collisions (FORMAN & ALEXANDER, 1998; FAHRIG & RYTWINSKI, 2009). Direct mortality can alter the demographic structure of wild animal populations (STEEN & GIBBS, 2004) and create sinks for local populations (NIELSEN *et al.*, 2006). Such changes can alter the structure and functionality of communities and ecosystems adjacent to roads (TROMBULAK & FRISSELL, 2000). The extent of these impacts depends on the characteristics of the roads, such as the road density, volume of traffic, landscape structure, proximity to protected areas, the different species of animals, and their natural histories (AMENT *et al.*, 2008; FRAIR *et al.*, 2008; FREITAS *et al.*, 2015).

2.5   Sustainable and green roads

The road development of a region is related to the transformation of the landscape (COFFIN, 2007). Currently, the institutions that finance this type of infrastructure request a sustainable approach in all stages of a road project (planning, design, construction, operation, and maintenance). According to the World Bank Group, development in all sectors must be done through the lens of social inclusion and

environmental sustainability to ensure that progress benefits the most vulnerable people and does not come at the expense of future generations (MONTGOMERY *et al.*, 2015; LOSOS *et al.*, 2019). Even the trend technological innovations are in search of new materials for paving, suitable designs with the landscape and renewable energy sources, which together can lead to sustainable road projects and free of greenhouse gas emissions (GHG) (NIJKAMP 1994; SODERLUND *et al.* 2008; FAIZ *et al.* 2012; ATTAHIRU *et al.* 2019).

The sustainability of roads is also evaluated based on their impact on wildlife. Mitigation strategies seek to reduce the effect of these impacts (CORLATTI *et al.*, 2009). These strategies can be aimed at modifying drivers' behavior by reduced speed limit, lights, and signs, or that of animals such as the installation of wildlife crossing structures (BEEBEE, 2013). However, the literature consensus is that a successful mitigation plan must be built based on the characteristics of the ecosystem where an existing road will be adapted, or a new one will be built (SEILER & HELLDIN 2006; GLISTA *et al.*, 2009; VAN DER REE *et al.*, 2015).

2.6   Environmental licensing and mitigation strategies in Brazil

In the PROFAS regulatory framework, it is stipulated that the highway management company to be licensed must fulfill environmental management activities and supervision and execution of environmental communication actions. Within the environmental program, the following are required: the environmental construction program (PAC, in Portuguese), the program for the prevention, monitoring, and control of erosive processes (PPMCPE, in Portuguese), and the program for the recovery of degraded areas (PRAD, in Portuguese). Together, the presentation of an Environmental Control Report (RCA, in Portuguese) is required, with the results of the PPMCPE, PRAD, and the road mortality monitoring programs of wildlife, environmental education, social communication, and environmental management must be recorded. Additionally, Article Nineteen of Decree 99.274 / 90 regulates the issuance of three types of licenses: the preliminary license (PL); the installation license (IL); and the operating license (OL). The toll road that executes the work must guarantee that compensatory activities are carried out (BECKMANN *et al.*, 2010; ABRA, 2012).

Another tool that Brazilian legislation has for protecting biodiversity is the National Action Plans for the Conservation of Endangered Species (PAN). These are public policies, agreed with society, that identify and guide priority actions to combat threats that put populations and species environments at risk and thus protect them. For example, the PAN of canids, felids, ungulates, giant-anteater, and Xenarthrans include road mortality mitigation among the main strategies (ICMBIO, 2021).

Some roads already have well-established environmental policies in the national territory and carry out mitigation measures that may be viable and effective in the Brazilian context. The structures built range from canopy structures (for arboreal animals) to fenced transects connected to underpasses and overpasses (FISCHER, 2001; CORLATTI *et al.*, 2009). Although Brazil has a consolidated history of road development, the lack of public resources has been a severe problem for road network maintenance. Nevertheless, the concession of the road administration to private entities has been a requisite strategy to allow the modernization and maintenance of the highways. Additionally, environmental legislation requires these toll roads to propose and implement an environmental strategy that mitigates biodiversity impacts. According to the Brazilian Association of Highway Concessionaires (ABCR, in Portuguese), 25,292 km of highways are operated by toll roads, 12% of the entire network (ABCR, 2021). In the authors' opinion, this strategy could be implemented in other roads since public and federal institutions do not have a sufficient budget allocation to carry out maintenance, monitoring, and implementation of the mitigation structures in the vast Brazilian road network.

2.7 Road mortality of wildlife in Brazil

Wildlife road mortality is of concern worldwide with alarming data in countries with available data, e.g., 340 million animal deaths annually in the USA, 32 million in Germany, 10 million in Spain, 5 million in Australia (SCHWARTZ *et al.*, 2020). In Brazil, wildlife road mortality estimates exceed 14 million (DORNAS *et al.*, 2012). In the state of São Paulo, a recent study detailed that on average, 39,500 wild mammals die each year, including threatened species such as manned wolf (*Chrysocyon brachyurus*), giant anteater (*Myrmecophaga tridactyla*), ocelot (*Leopardus pardalis*), and puma

(*Puma concolor*) (ABRA *et al.*, 2021). Therefore, we carried out a bibliographic survey to detail biodiversity loss due to vehicular collisions in Brazil between 1988 and 2021. Table 1 describes the keyword combinations used in the searches. Searches were conducted on EBSCOHost, PUBMED, Google Scholar, and Scielo. In addition, to obtain local studies, the databases of the public universities of the 27 federative units of Brazil were used in search of monographs, dissertations, and doctoral theses.

Table 1. Keyword combinations used in the literature review

Topic	Keywords
Brazil and roads	History + Roads + Brazil + Land Transport
Roadkill	Roadkill + UF + Monitoring + Road Ecology

In the literature review, we found 92 documents, being 66 scientific articles (71.7%), 16 monographs/dissertations/theses (17.4%), 8 conference abstracts (8.7%), 1 book chapter (1.1%) and 1 technical report (1.1%). One of the articles corresponds to a compilation of information that includes 70 studies (six were excluded as they were already represented). The first published study covering the quantification of road mortality in wild species was carried out by Novelli et al. (1988) (Figure 1). This study describes the diversity of wild birds killed by Motor Vehicle Collisions (MVC) throughout a road in the Rio Grande do Sul (RS). Studies were obtained from 24 of the 27 federative units in the country, with the Rio Grande do Sul (RS), Goias (GO), Sao Paulo (SP), Minas Gerais (MG), and Santa Catarina (SC) being the FUs with the highest representation (Figure 2). Only in Amapá (AP), and Rio Grande do Norte (RN) no studies were found. In total, the loss of wildlife by MVC in these studies was 114,329 individuals distributed as follows: mammals 74,685 (65.3%), birds 16,848 (14.7%),

reptiles 12,604 (11.0%), amphibians 8,348 (7.3%), 1,844 specimens were not identified.

In the 92 scientific works, different methodologies are described to carry out road monitoring. These monitoring include vehicles at speeds between 40-6 km/hour, motorcycles, bicycles, and even foot. Foot surveys are much more effective in identifying small-sized specimens (e.g., small mammals, reptiles, and amphibians). However, it is impossible to carry out long transects (> 5km). On the contrary, it is possible to cover greater distances using vehicles, but small-sized animals can be underestimated. In total, in these works, 18,376.21 km of roads were monitored. The most frequent experimental designs were: 1 day/month (15%), 1 day/week (12%), 2 days/week (10%). Only two (2%) studies conducted daily monitoring.

Figure 1. Temporal distribution of the studies of road mortality of wildlife in Brazil between 1988 and 2021.

	1988	2004	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
N	1	1	6	3	3	5	5	5	10	8	8	16	6	3	5	5	1	5
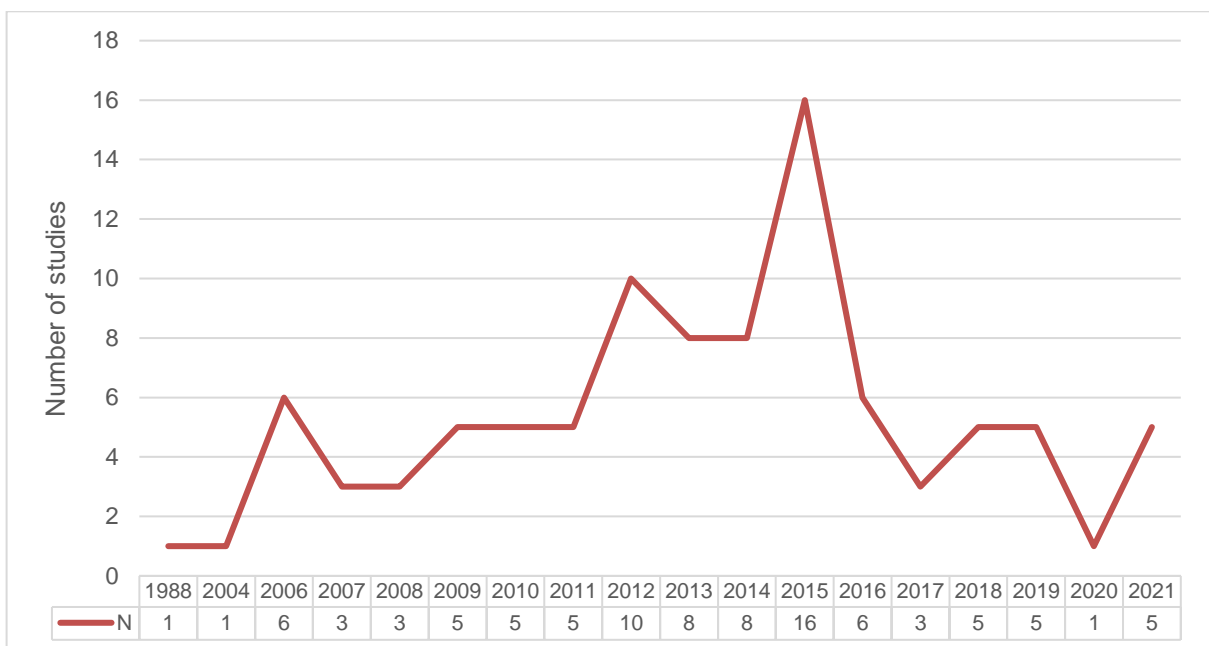
Figure 2. Distribution of studies according to frequency in the states of Brazil.
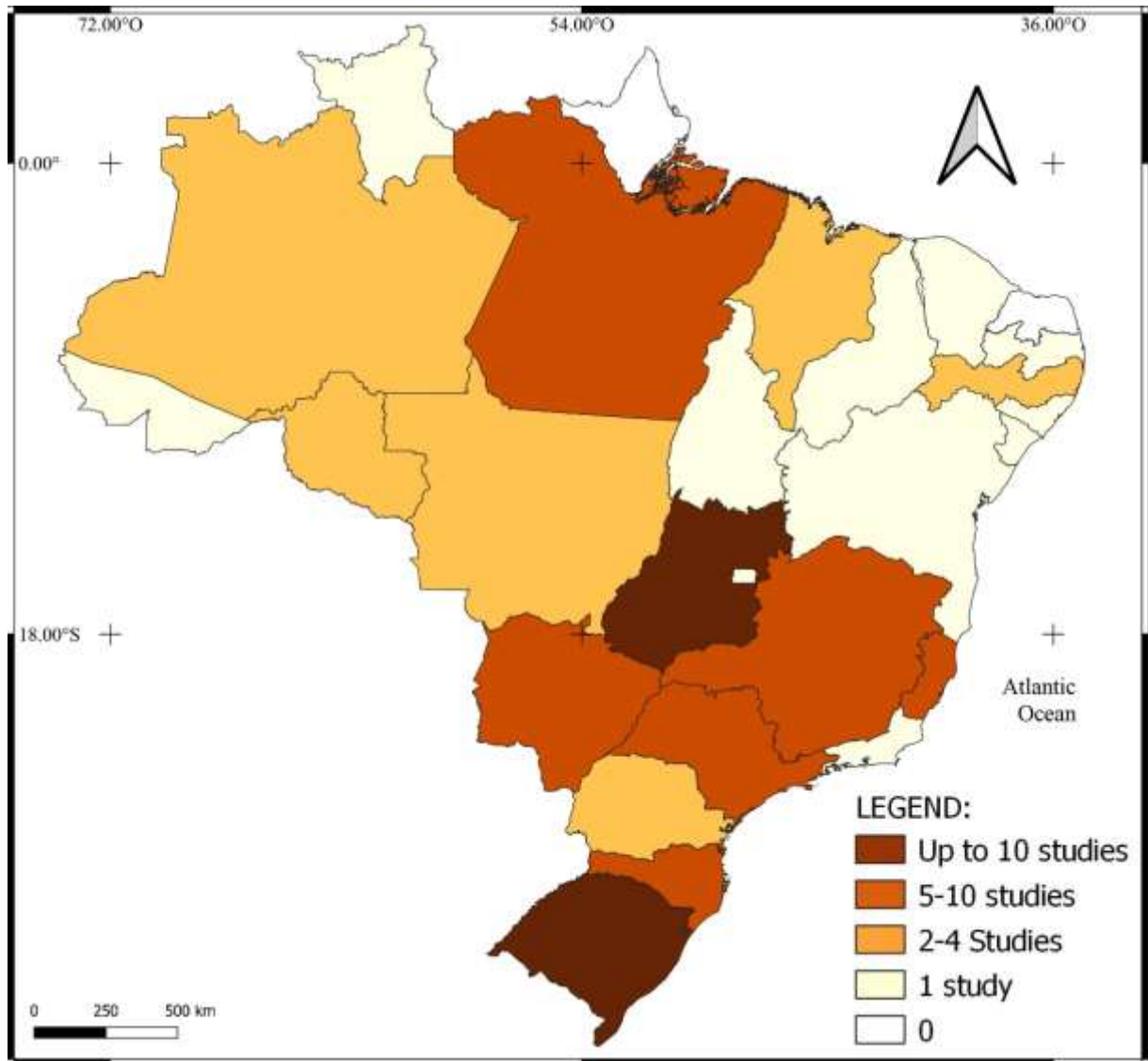
2.8 Disease surveillance using wildlife mortality on roads in Brazil

Additionally, according to the Law 6.259 of 1975, Regulation No. 782 of 2017 of the Ministry of Health details the public health events (PHE). This document includes the death of non-human primates, wild birds, and canids and wildlife mortality due to an undetermined cause. Consequently, these events must be notified to trigger epidemiological investigations to clarify the cause of death and implement surveillance of infectious diseases, e.g., west Nile virus, rabies, or yellow fever (MINISTÉRIO DA SAÚDE, 2017). In general, researchers have a lag in studies about the mortality of

wildlife on the roads. Therefore, the studies mainly describe the number of dead animals and carry out temporal and spatial studies seeking to describe the environmental characteristics and the critical points of aggregation. Nevertheless, there are some examples of research in which road mortality were used to investigate other aspects, especially health issues. These studies include the description of nutritional characteristics (PASA *et al.*, 2020), diversity of ectoparasites (SPOLIDORIO *et al.*, 2014), endoparasites (OLIVEIRA *et al.*, 2020; BENATTI *et al.*, 2021), the occurrence of viral (CALABUIG *et al.*, 2019), bacterial (CALCHI *et al.*, 2020), fungal agents (RICHINI-PEREIRA *et al.*, 2008; NAVAS-SUÁREZ *et al.* 2021 [**This study chapter 3**]), and even pesticides (MEDICI *et al.*, 2021). This information demonstrates the importance of wildlife mortality on the road since it is an easily accessible source of information, comprising higher species diversity, including threatened ones. In addition, according to Brazilian legislation, some species of wild animals, such as neotropical primates, must be investigated to determine the presence of the yellow fever virus. However, despite the lack of regulation, the investigation of pathogens in the other species is significant to public health and the authors believe this practice should be implemented whenever possible.

2.9   Final considerations

The historic of social and economic development in Brazil due to highways is evident. Still, this road development caused several primary and secondary environmental impacts. The development of road infrastructure in Brazil dates back to the arrival of the royal family in 1808. This prompted the inhabitants of colonial Brazil to ask the king to develop the road infrastructure (precarious at this time); However, it was only until 1859 that the country's first public transportation system was inaugurated in Rio de Janeiro.

We identify three moments in the country's history (1928, 1938, 1956) in which government decisions favored the development of road infrastructure to the detriment of railways and waterways. In general, road development in Brazil implies that it currently has one of the most extensive road networks globally, however, due to the

lack of a national budget, the average quality of the roads is low. In addition, this implies that the transport of commodities and passengers are dependent on roads, which are a more expensive and lesser efficient means of transport than trains and waterways.

Thanks to advances in environmental legal frameworks in the country, such as the legislation that creates environmental licensing, several road projects considered the environmental impacts and created forms of mitigation and compensation. Regarding the specific impact of wildlife road mortality, several scientific studies have been published on this topic, with an emphasis on the southeast and central west areas of Brazil.

Currently, several highways have mitigation structures to reduce the roadkill impact, such as fences and wildlife passages, but many current highway projects still put biodiversity at risk, such as the BR-319 highway, which has the potential to increase the arc deforestation and the continuation of the BR-364 highway connecting Acre state with Peru. These two highways, located in the Amazon Biome, put the integrity of the Amazon Forest, conservation units and territories of traditional communities at risk. We believe the implementation of mitigation strategies in a much broader scale, especially including roads within the most biodiversity areas of the country, as the Amazon basin, Pantanal and Cerrado, is a necessary and urgent public policy to contribute for the Brazilian biodiversity.

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Supplemental table 1. Brazilian legal framework on roads and environmental conservation

Law	Character	Status
12.619/2012	Regulation of truck drivers' working hours.	Active
12.379/2011	Provisions on the National Road System.	Active
11.442/2007	Regulations for cargo transportation.	Active
10.233/2001	Creation of the National Land Transport Agency (ANTT).	Active
9.985/2000	Creation of the National System of Nature Conservation Units.	Active
9.277/1996	Authorization by the Union (Federation) to delegate the administration and operation of federal highways to municipalities, states and the Federal District.	Active
7.092/1983	Creation of the National Register of Road Transport of Goods (RTB).	Revoked
6.813/1980	Regulation of Cargo Transport as a private activity of Brazilian autonomous carriers.	Revoked
6.938/1981	National Environmental Policy Law	Active
Ordinance N289/2013	Provides for procedures for environmental licensing of highways and environmental regularization of federal highways.	Active
R 3056/2009	Qualification for the road freight carrier on behalf of third parties for remuneration.	Active
PM 10/1993	Creation of the federal highway concessions program.	Active
D47.400/2002	Regulation of environmental licensing on roads, in the Sao Paulo State.	Active
D99.274/1990	Regulation of environmental licenses issued by the Government.	Active
R CONAMA 237/1997	Licensing system as an instrument of environmental management, established by the National Environmental Policy.	Active
NR-11	Safety procedures in activities involving transport, storage, handling and movement of products and materials in national territory.	Active

3   CHAPTER II: UNDERSTANDING TRAUMATIC INJURIES BY MOTOR VEHICLE COLLISIONS (MVC-TI) IN NEOTROPICAL WILD MAMMALS.

3.1 **PATHOLOGICAL FINDINGS IN LOWLAND TAPIRS (*TAPIRUS TERRESTRIS*) KILLED BY MOTOR VEHICLE COLLISION IN THE BRAZILIAN CERRADO**

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3.1.1 **ABSTRACT**

The lowland tapir (*Tapirus terrestris*) is the largest land mammal in South America. The species faces steady population decline due to poaching, habitat loss and fragmentation, road-kill, pesticide pollution, competition with domestic livestock and fires, among other threats. The lowland tapir is currently listed as vulnerable to extinction. Little information is available about natural disease processes for the species. This study aimed to report the pathological findings recorded in a cohort of 35 lowland tapirs killed by motor vehicle collision (MVC) on highways of Mato Grosso do Sul State, Brazil, between 2015 and 2018. The main gross pathological findings were those associated with MVC, primarily involving skeletal fractures and internal multiorgan damage with extensive bleeding and/or severe central nervous system injury. The most prevalent concurrent histopathological findings, unrelated to the cause of death, were: adrenal gland degeneration, necrosis and loss of fascicular and reticular cells with replacement fibrosis and cortical atrophy (9/15; 60%); interstitial pneumonia (20/34; 59%); glossitis (9/24; 38%); pulmonary anthracosis (12/34; 35%); colitis (9/28; 32%); and cholangitis/pericholangitis (9/35; 26%). The aetiopathogeneses and clinicopathological significance of some of these findings are unclear; however, parasitic infections appear to be common. Our results highlight the importance of wildlife health information obtained through the study of carcasses of roadkills.

Keywords: adrenocortical fibrosis; lowland tapir; road-kill; wildlife pathology

3.1.2 **INTRODUCTION**

The Cerrado biome comprises 2 million km2, representing approximately 23% of Brazilian land surface. In 2002, it was estimated that at least 40% of the Cerrado area had been already cleared (Sano et al., 2010). The major threats to the Cerrado are the expansion of the agricultural frontier, road construction, deforestation and

urbanization (Klink and Machado, 2005). In the last 40 years, many highways have been built aiming to facilitate the transport of agricultural products to large urban centres and seaports for export (Miranda et al., 2017). Motor vehicle collision (MVC) or road-kill has been recognized to affect almost all vertebrate species of the Cerrado, including lowland tapirs (*Tapirus terrestris*) (Ferreira da Cunha et al., 2010; Caceres et al., 2010, 2012; Ascensão et al., 2017).

Tapirs (order Perissodactyla; family Tapiridae) are large herbivores that inhabit tropical forests in South and Central America and Southeast Asia. The lowland tapir, one of the four living tapir species worldwide, is currently listed as vulnerable to extinction, as it shows steady population decline in different biomes in Brazil due to poaching, habitat loss and fragmentation, road-kill, pesticide pollution, competition with domestic livestock and fires (Medici et al., 2018). The species has a large distribution range from northern Colombia to the east of the Andes throughout most of tropical South America, including 11 countries (Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname and Venezuela) (IUCN, 2008).

There is limited knowledge about natural diseases affecting free-ranging tapirs. The information available in the literature is mostly related to captive individuals and includes: musculoskeletal disease (e.g. inter- and intraspecific trauma, lameness, mandibular abscessation, degenerative joint disease), gastrointestinal disease (e.g. intestinal volvulus, colonic incarceration, gastric and colonic impactions), metabolic disease (e.g. iron and copper deficiency, haemochromatosis, hypovitaminosis E), respiratory disease (e.g. pneumonia, tuberculosis, pleural adhesions), reproductive disease (e.g. genitourinary tract infection, metritis, leiomyosarcoma), dental disease (e.g. fractures of incisors, molar abscess), cutaneous disease (e.g. vesicular skin syndrome, sarcoptic mange), and renal disease (e.g. renal failure), among others (Quse and Fernandes-Santos, 2014; Zimmerman and Hernandez, 2014; Duncan, 2018).

Some of the reported infectious agents in tapirs include viruses (foot and mouth disease virus, bluetongue virus, porcine parvovirus, infectious bovine rhinotracheitis virus, herpesvirus, encephalomyocarditis virus, eastern equine encephalitis virus, Venezuelan equine encephalitis virus and western equine encephalitis virus), bacteria

(Mycobacterium spp., Salmonella spp., Leptospira interrogans, Clostridium spp.), fungi (Microsporum spp., Trichophyton spp., Coccidioides spp.) and protozoa (Giardia spp.) (Quse and Fernandes-Santos, 2014; Zimmerman and Hernandez, 2014; Duncan, 2018). In contrast, there are few studies reporting health issues of wild tapir populations and most of them are based on serological surveys (Hernandez-Divers et al., 2005; Furtado et al., 2010; Medici et al., 2014). Further studies focused on confirming infectious aetiologies by direct laboratory methodologies (e.g. immunohistochemistry, in-situ hybridization, polymerase chain reaction) and identifying pathogenetic mechanisms in free-ranging tapirs are much needed. A review of available clinical and pathological data, together with laboratory diagnostic techniques, is provided in Supplementary Table 1.

Necropsy examinations offer unique opportunities to identify pathological processes in more detail than occurs in studies of health surveillance of captured animals. Gross and microscopical examination of tissues may produce valuable information allowing identification of novel pathological processes and understanding of the pathogenesis and/or functional disturbances of a disease (Wobeser, 1996; Quse and Fernandes-Santos, 2014). The aim of the present study was to report the pathological findings in a set of 35 lowland tapirs killed by MVC on the highways of Mato Grosso do Sul State, Brazil, between 2015 and 2018.

3.1.3 MATERIALS AND METHODS

Study Area and Data Collection

Lowland tapir carcasses were located during a systematic tapir road-kill monitoring surveillance programme in the Mato Grosso do Sul state, Brazil (Lowland Tapir Conservation Initiative [LTCI], 'Instituto de Pesquisas Ecologicas' [IP^E]). The programme monitored approximately 1,185 km of paved highways across the state, representing 15% of the total state highway network. For 3 years (2015e2018), the highways were monitored for tapir road-kill every 15 days. Fresh tapir carcasses

(typically up to 24 h after death) were subjected to detailed necropsy examinations. For each tapir carcass evaluated, the following data were recorded: date, GPS coordinates, municipality, highway, total body length, sex, age class, nutritional status (cachectic, poor, moderate or good) and decomposition status (fresh, initial autolysis, moderate autolysis or advanced autolysis) (modifications from the categories proposed by Vass et al., 2002).

Pathological Examination

Necropsy procedures were performed according to Quse and Fernandes-Santos (2014). External traumatic injuries were classified into four categories, according to the anatomical region affected: head and neck (HN), thorax (TX), abdomen/pelvis (AP) and extremities (EX) (Kolata et al., 1974). Representative tissue samples of skin, skeletal muscle, tongue, oropharynx, tonsil, salivary glands, oesophagus, stomach, small and large intestines, liver, gallbladder, pancreas, larynx, trachea, lung, heart, great vessels, thymus, spleen, lymph nodes (mandibular, pharyngeal, prescapular, mediastinal, bronchial, lumbar, mesenteric and popliteal), kidney, ureters, urinary bladder, urethra, thyroid and parathyroid glands, adrenal glands, diaphragm, cerebrum, cerebellum, spinal cord, eye, mammary gland, testicle, ovary, uterus, epididymis and prostate were collected and fixed in 10% neutral buffered formalin. Tissue samples were processed routinely and embedded in paraffin wax. Sections (5 mm) were stained with haematoxylin and eosin (HE). Additional histochemical and immunohistochemical techniques to better characterize the lesions in selected cases included: periodic acideSchiff (PAS; for fungi and basement membranes), Grocott-Gomori's methenamine silver (GMS; for fungi), ZiehleNeelsen (ZN; for mycobacteria), Gram/Twort (for bacteria), Masson's trichrome (for collagen/fibrosis) and CD3 immunohistochemistry (IHC) (for T lymphocytes). For IHC, a rabbit polyclonal anti-CD3 antibody (1 in 100 dilution; A0452; Dako, Carpinteria, California, USA) was used. Antigen retrieval (EDTA pH 9.0, pressure cooker) was followed by blocking of endogenous peroxidase activity and blocking of nonspecific binding with normal rabbit serum. The primary antibody was incubated overnight (18 h, 4C). Amplification and visualization were achieved by use of the HiDef Detection—

HRP Polymer System (Cell Marque, Rocklin, California, USA) followed by 3, 30 diaminobenzidine (DAB D-5637; Sigma, St. Louis, Missouri, USA) as chromogen and counterstaining with Harris' haematoxylin. Normal human lymph node was used as positive control. Tissue sections in which the primary antibody was replaced by non-immune homologous serum served as negative controls. When helminths were observed, these were collected and identified according to protocols of the Department of Parasitology, Institute of Biosciences, University of São Paulo State, São Paulo, Brazil.

3.1.4 **RESULTS**

A total of 35 tapir carcasses were included in the study. Evaluated individuals were separated into four age classes: fetus in the last third of gestation (1/35; 3%), juvenile (3/35; 9%), subadult (8/35; 23%) and adult (23/35; 65%). Sex distribution was male (20/35; 57%) and female (15/35; 43%). All animals were in good body condition. The detailed general information of these animals is recorded in Table 1.

All tapirs died as a result of severe traumatic lesions following MVC, primarily involving skeletal fractures and internal multiorgan damage with extensive bleeding and/or severe central nervous system (CNS) injury. The distribution of external traumatic injuries was: TX and AP (25/35; 71%, each) and HN and EX (18/35, 51%, each). Seven animals had concomitant injuries in all anatomical regions (Figs. 1 and 2). MVC-associated injuries included rupture or disruption of parenchymal organs (n = 32; spleen, n = 22; lungs, n = 17; stomach, n = 18; liver, n = 16; tongue, n = 9; heart/brain, n = 8; intestines, n = 7 with/without release of digesta/faeces), haemoperitoneum (n = 32), cutaneous abrasions (n = 32), skeletal fractures (n = 28; ribs, n = 17; pelvis, n = 12; jaw, n = 11; femur/tibia, n = 9; neurocranium, n = 8), haemothorax (n = 28), haemorrhage in parenchymal organs (n = 23), contusion of parenchymal organs (n = 20) and pulmonary oedema (n = 19), among others. Prevalent gross findings unrelated to trauma were the presence of ticks (Family

Ixodidae; n = 31), abundant gastric ingesta (n = 14) and hepatomegaly (n = 10). Main gross findings are recorded in Table 2.

Relevant microscopical findings are recorded in Table 3. MVC-associated findings were confirmed microscopically, including multiorgan haemorrhage and rupture (lung, spleen and liver). The most prevalent concurrent pathological findings unrelated to the cause of death were (in decreasing order): adrenal gland degeneration, necrosis and loss of fascicular and reticular cells with replacement fibrosis and cortical atrophy (60%, Fig. 3); interstitial pneumonia (59%, Fig. 4); chronic enteritis (52%, Fig. 5); chronic tracheitis (42%); glossitis (38%); pulmonary anthracosis (35%); chronic colitis (32%, Fig. 6); and cholangitis/pericholangitis (26%, Fig. 7). Various known and novel pathological findings or disease processes were observed, namely chronic granulomatous lymphadenitis (21%, Fig. 8), chronic orchitis (17%, Fig. 9), nodular proliferative gastritis associated with Physocephalus spp. (9%, Fig. 10), proliferative endarteritis with stenosis in the vasculature of the intrinsic glossal muscle (9%, Fig. 11), endocarditis/myocarditis (9%), lymphocytic meningitis (3%), a large focal ectopic haemopoietic nodule mainly comprised of CD3+ lymphocytes in the liver of a fetus (3%) and chronic mastitis in two cases (100%).

Chronic enteritis characterized by minimal to moderate lymphoplasmacytic inflammatory infiltrates was observed in 16 tapirs and eosinophilic colitis was diagnosed in nine animals. All of these animals had mild to moderate numbers of ciliated trophozoites. Trophozoites measured approximately 50-150 mm in length, with cilia arranged in rows on the outer surface and a macronucleus and a micronucleus. Most ciliates were located among the intestinal villi or within the colonic crypts (Fig. 6). Occasionally, a few ciliates were adherent to the villi with damaged epithelium. Rare ciliated trophozoites were seen in animals without inflammatory changes. Metazoan parasites were observed in 14 cases. According to the region, they were distributed as follows: caecum (n = 9), stomach (n = 5), small intestine (n = 2) and abdominal cavity (n = 1). Five species were identified: *Cladorchis asper* (caecum; n = 3), *Cladorchis pyriformis* (cecum, n = 2), *Kiluluma longipene* (stomach, n = 2; small intestine, n = 1; caecum, n = 4), *Murshidia monosticha* (small intestine, n = 1; abdominal cavity, n = 1) and *Physocephalus* spp. (stomach, n = 3). Detailed gross and microscopical findings in these animals are recorded in Supplementary Table 2.

Table 1. General data of 35 lowland tapirs (*T. terrestris*) included in this study

Number	Date	M	Road	Age	Sex	BW (kg)	DC	TC
1	Feb 15	M1	A	Adult	Female	200-250	3	TX, AP
2	Feb 15	M1	A	Gestational	Female	4	2	AP
3	Sep 15	M2	B	Subadult	Female	200-250	2	TX, AP
4	Dec 15	M3	C	Adult	Female	200-250	2	HN, TX
5	Feb 16	M4	D	Adult	Female	180-200	2	HN, TX, AP, EX
6	Feb 16	M5	A	Adult	Female	180-200	2	HN, TX
7	Mar 16	M5	A	Juvenile	Female	180-200	2	TX, AP, EX
8	Mar 16	M1	A	Subadult	Female	180-200	2	HN, TX, EX
9	Apr 16	M1	A	Subadult	Female	180-200	2	TX, AP, EX
10	May 16	M5	A	Adult	Male	200-250	2	HN, TX, AP, EX
11	May 16	M5	B	Adult	Male	200-250	2	TX, AP
12	Jun 16	M6	C	Subadult	Female	200-250	3	HN, TX, AP, EX
13	Jul 16	M5	A	Juvenile	Male	100-120	2	AP, EX
14	Jul 16	M5	A	Adult	Female	200-250	2	HN, TX, AP, EX
15	Jul 16	M6	C	Adult	Male	200-250	2	EX
16	Jul 16	M5	A	Subadult	Male	180-200	2	HN, TX, AP
17	Jul 16	M5	A	Subadult	Male	180-200	3	HN, AP, EX
18	Jul 16	M5	A	Adult	Male	200-250	2	AP, EX
19	Sep 16	M1	A	Adult	Male	150-180	2	TX, AP, EX
20	Oct 16	M7	5	Adult	Male	200-250	2	TX, EX
21	Oct 16	M5	A	Adult	Male	200-250	2	TX, AP
22	Oct 16	M5	A	Adult	Male	200-250	2	HN, TX, AP, EX
23	Nov 16	M5	A	Subadult	Male	180-200	2	HN, TX, AP, EX
24	Nov 16	M5	A	Adult	Female	200-250	2	HN, EX
25	Nov 16	M8	D	Adult	Male	180-200	2	PA
26	Feb 17	M3	C	Juvenile	Male	70-100	2	HN, TX, AP, EX
27	Apr 17	M5	A	Subadult	Female	100-150	2	HN, TX, EX
28	Apr 17	M3	C	Adult	Male	150-180	2	HN
29	May 17	M3	C	Adult	Female	200-250	2	HN, TX
30	May 17	M5	A	Adult	Male	200-250	3	HN
31	Dec 17	M3	C	Adult	Female	200-250	2	HN, TX, AP
32	Dec 17	M6	C	Adult	Male	200-250	2	TX, AP
33	Jun 18	M3	D	Adult	Male	150-200	2	TX, AP
34	Jul 18	M6	C	Adult	Male	200-250	2	TX, AP
35	Jul 18	M3	D	Adult	Male	200-250	3	AP

M, municipality; M1, Nova Andradina; M2, Rio Brilhante; M3, Ribas do Rio Pardo; M4, Terenos; M5, Nova Alvorada do Sul; M6, Campo Grande; M7, Novo Horizonte do Sul; M8, Dois Irm~aos do Buriti; A, BR267; B, MS145; C, MS040; D, BR262; E, MS147. BW, body weight. DC, decomposition code (2, fresh; 3, moderate autolysis); TC, trauma classification; HN, head and neck; TX, thorax, PA, abdomen, and pelvis; EX, extremities.

Table 2. Gross findings in 35 road-killed lowland tapirs (*T. terrestris*)

Gross finding	Af/Ev (%)
Rupture of parenchymal organs*; haemoperitoneum*	32/35 (91)
Tick infestation (primarily argasidosis)	31/35 (89)
Cutaneous abrasion*	29/35 (83)
Skeletal fractures*; haemothorax*	28/35 (80)
Haemorrhage of parenchymal organs*	23/35 (66)
Contusion of parenchymal organs*; faeces in abdominal cavity*	20/35 (57)
Pulmonary oedema*	19/35 (54)
Gastric content in abdominal cavity*	18/35 (51)
Cutaneous laceration*	17/35 (49)
Stomach dilated by ingesta	16/35 (45)
Congestion of parenchymal organs*; multifocal haematomas*	14/35 (40)
Hepatomegaly*	10/35 (29)
Discoloured (yellow) adrenal gland	9/35 (26)
Rectal prolapse*	8/35 (23)
Intestinal endoparasitosis	7/35 (20)
Adrenomegaly; acute passive hepatic congestion	5/35 (14)
Haemopericardium*; partial evisceration*	4/11 (11)
Nail avulsion*; dental wear and/or fractures	3/35 (9)
Distended urinary bladder; exophthalmia*; hepatic abscesses; scapulohumeral luxation*; gastric nematodiasis	2/35 (6)
Cardiomegaly; hydroperitoneum; interstitial nephritis; submandibular lymphadenomegaly; caecal trematodiasis	1/35 (3)

Af, affected; Ev, evaluated.
*Findings associated with motor vehicle collision.

Table 3. Microscopical findings in 35 road-killed lowland tapirs (*T. terrestris*) included in this study

Microscopical finding	Af/Ev (%)
Degeneration, necrosis, and loss of fascicular and reticular cells with replacement fibrosis and cortical atrophy	9/15 (60)
Chronic interstitial pneumonia	20/34 (59)
Acute pulmonary haemorrhage*	19/34 (56)
Acute splenic haemorrhage*	17/31 (55)
Chronic enteritis	16/30 (52)
Lymph node haemorrhage*	9/19 (47)
Chronic tracheitis	11/26 (42)
Superficial glossitis	9/24 (38)
Pulmonary anthracosis	12/34 (35)
Chronic colitis	9/28 (32)
Pulmonary emphysema*	11/34 (32)
Acute renal haemorrhage*	10/34 (29)
Chronic cholangitis/pericholangitis	9/35 (26)
Splenic congestion*	8/31 (26)
Pulmonary oedema*	8/34 (24)
Paracortical lymphoid reactive hyperplasia; chronic granulomatous lymphadenitis	4/19 (21)

Af, affected; Ev, evaluated.
*Findings associated with motor vehicle collision.

Fig. 1. (Case 23) Adult road-killed lowland tapir (*Tapirus terrestris*) with marked, multifocal cutaneous abrasions, deviated (fractured) right tibia, abdominal externalization of the small intestine and multifocal external bleeding.

Fig. 2. (Case 31) Severe thoracic and abdominal trauma with gastrointestinal rupture and released ingesta and faeces, having protruded through the ruptured diaphragm and displacing the right lung lobes craniodorsally. There is haemothorax due to multiple cardiac and lung fractures (not evident in this image) and extensive subcutaneous haemorrhage in the brisket area.
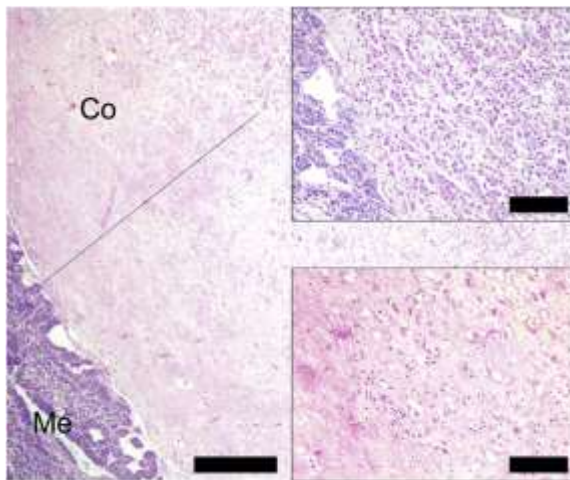
Fig. 3. (Case 15). The zona reticulata and fasciculata epithelial cells of the adrenal glands are replaced by fibrous connective tissue (straight line indicates the extent of fibrosis). HE. Bar, 200 mm. Upper inset: normal adrenal gland from an age class-matched 'normal' tapir (case 7). HE. Bar, 100 mm. Lower inset: degenerating cortical cells of zona reticulata and fasciculata with extensive loss and abundant fibrosis. HE. Bar, 100 mm.
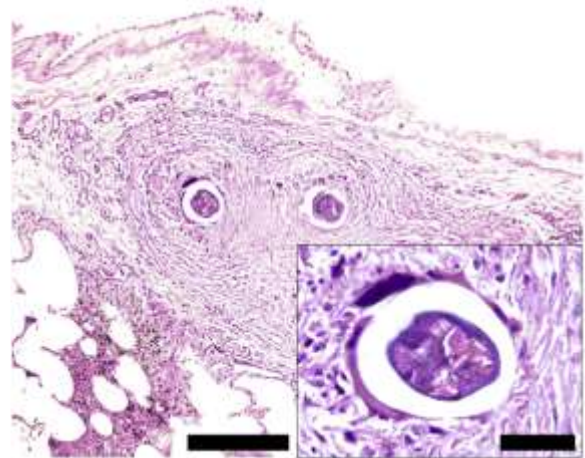
Fig. 4. (Case 15) The pleura is focally expanded by a chronic granulomatous and eosinophilic inflammatory nodule with fibrosis and multiple central transverse sections of adult nematodes. HE. Bar, 200 mm. Inset: detail of strongyloid nematode within the inflammatory nodule. HE. Bar, 50 mm.
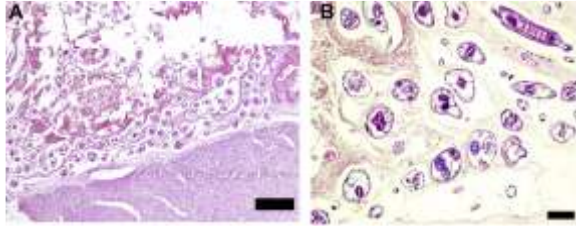
Fig. 5. (Case 23) (A) The colonic mucosa is invaded by numerous nematodes without evident inflammatory reaction. HE. Bar, 200 mm. (B) Detail of intramucosal nematodes. There is some autolysis-associated loss of cellular detail in this field. HE. Bar, 50 mm.
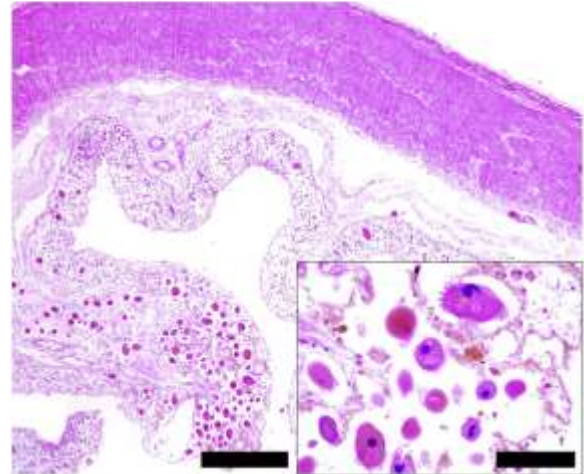
Fig. 6. (Case 31) The colonic mucosa is invaded by numerous ciliated protozoan trophozoites. HE. Bar, 500 mm. Inset: protozoan trophozoites with cilia arranged in rows on the outer surface and a macronucleus. HE. Bar, 100 mm.
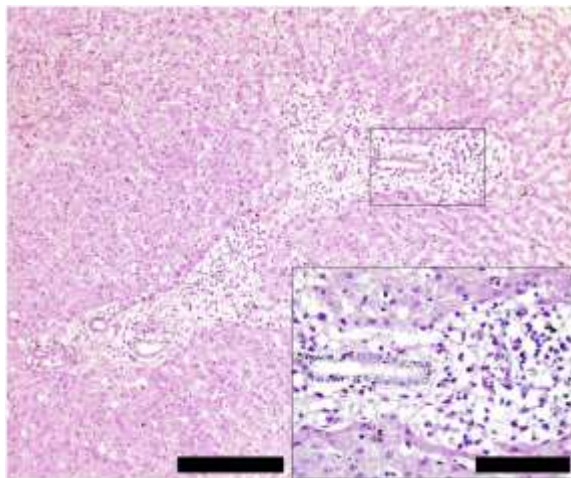
Fig. 7. (Case 7). A portal tract is infiltrated by pleocellular inflammatory cells. HE. Bar, 200 mm. Inset: a moderate number of eosinophils and fewer lymphocytes, plasma cells and macrophages expand the portal triad. HE. Bar, 100 mm.
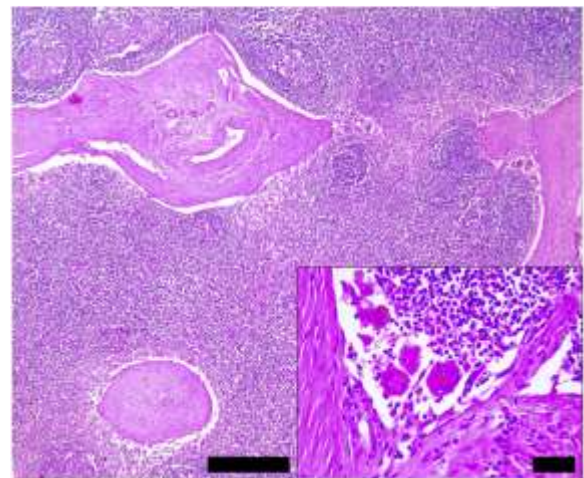
Fig. 8. (Case 25). Lymph node with granulomatous inflammatory cells primarily confined to sinus and adjacent areas. HE. Bar, 200 mm. Inset: a subcortical/marginal sinus contains reactive macrophages, binucleated macrophages and multinucleate giant cells. HE. Bar, 50 mm.
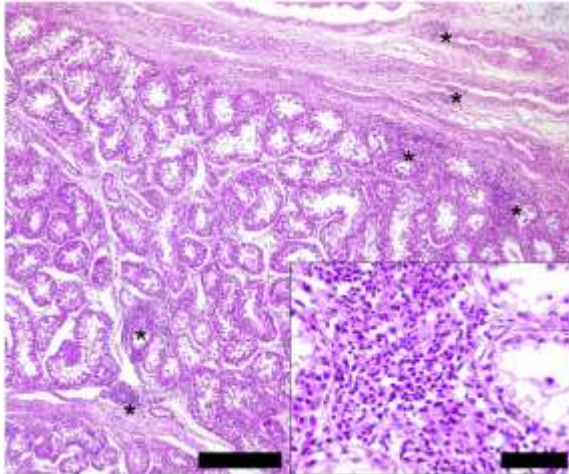
Fig. 9. (Case 32) The tunica albuginea and testicular parenchyma are multifocally infiltrated by mononuclear inflammatory cells (asterisks). HE. Bar, 200 mm. Inset: focus of lymphoplasmacytic inflammation expanding the testicular parenchyma and infiltrating the seminiferous tubules. HE. Bar, 50 mm.
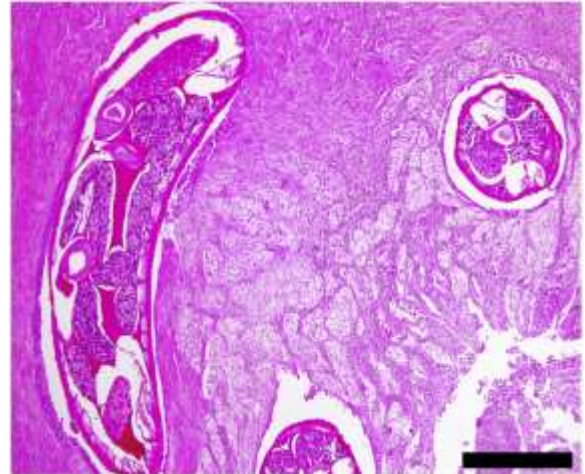
Fig. 10. (Case 18). The gastric mucosa and submucosa contain three sections of nematodes surrounded by chronic pleocellular inflammatory cells and fibroplasia. There is gastric mucous cell metaplasia/hyperplasia in the overlying epithelial lining. The nematodes show typical pseudocelomic eosinophilic material as well as large lateral cords, digestive, and reproductive structures. HE. Bar, 200 mm.
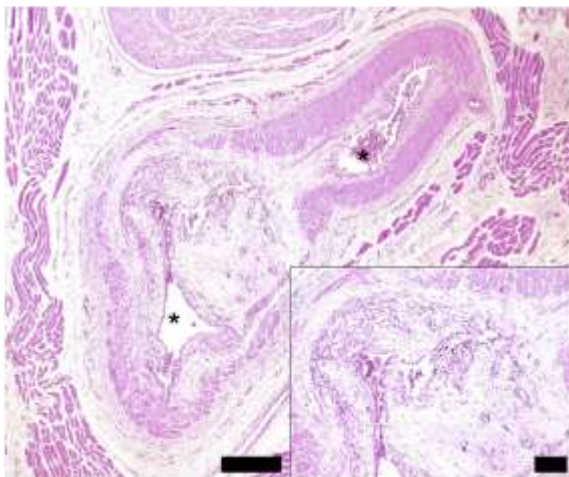
Fig. 11. (Case 25) A medium calibre artery within glossal intrinsic musculature is stenosed by redundant intraluminal fibrovascular proliferation. HE. Bar, 200 mm. Inset: detail of chronic intraluminal fibrovascular proliferation with remodelling and stenosis. HE. Bar, 100 mm.

3.1.5 **DISCUSSION**

Mortality by MVC represents an important threat to lowland tapirs in Brazil, particularly in the Cerrado biome (Medici and Desbiez, 2012; Medici et al., 2018). The LTCI has recorded almost 500 tapir carcasses along 34 highways in the Mato Grosso do Sul State from 2013 to 2019 (83 tapirs per year) (Medici et al., 2016). This level of impact may lead to tapir population decreases and local extinction as well as a loss of reproductive stock within affected populations.

We found a variety of gross and microscopical findings unrelated to MVC in this cohort of tapirs. Adrenal gland disease characterized by varying degrees of degeneration, loss and fibrous replacement of epithelial cell of the reticulata and fasciculata regions, was highly prevalent (60%). In some instances, fibrosis replaced more than 50% of the cortex; glomerulata cells and medullary cell populations appeared spared in all cases. To the authors' knowledge, such a naturally occurring pathological finding with such high prevalence has not been documented in the human or veterinary literature dealing with adrenal gland pathology. Although the aetiopathogenesis and clinicopathological relevance of this finding are unknown, it is reasonable to think that it could play a role in endocrine dysregulations, including adrenocortical insufficiency. Five main pathogenetic mechanisms might be considered: (1) a direct toxic effect (e.g. by a xenobiotic substance such as a toxic plant or chemical pollutant); (2) chronic inflammation of unknown infectious/non-infectious nature; (3) chronic adrenocortical overstimulation; (4) radiation damage; or (5) degenerative genetic derangement (Vilar and Tullner, 1959; Rotterdam and Dembitzer, 1993; Carvalho et al., 2015; Levine, 2018). Only one case had scattered foci of lymphoplasmacytic inflammation, so a potential association with a chronic inflammatory process was not sufficiently supported. Further studies are needed to elucidate the aetiopathogenesis and clinicopathological relevance of this prevalent lesion.

Eosinophilic interstitial pneumonia was described in 20 tapirs. This lesion is likely of parasitic nature (adults and migrating nematode larvae); however, we only observed Strongylida nematodes in the subpleural pulmonary parenchyma in one

case, associated with a granulomatous response, as well as interstitial eosinophilic pneumonia. Further histological identification of the nematodes was not achieved. Lungworms of domestic animals typically involve genera of the order Strongylida, namely *Dictyocaulus*, *Angiostrongylus* and *Metastrongylus*; another possibility in these cases would be hypersensitivity reactions (Caswell and Williams, 2015). The aetiology of this lesion remains unknown in these cases.

Chronic enteritis characterized by minimal to moderate lymphoplasmacytic inflammatory infiltrates was observed in 16 tapirs and eosinophilic colitis was diagnosed in nine animals. All of these animals had mild to moderate numbers of ciliated trophozoites. In domestic animals, three genera of ciliated protozoa associated with eosinophilic colitis have been reported: *Buxtonella* spp. in cattle (Urman and Kellky, 1964), *Polymorphella cycloposthium* in horses (French et al., 1996) and *Balantidium coli* in man, pigs and horses (Schuster and Ramirez-Avila, 2008; Uzal et al., 2015). While previous coproparasitological studies indicated *Balantidium* spp., *Eimeria* spp. and *Giardia* spp. oocysts may be normal enteric flora in tapirs, these protozoa may be associated with diarrhoea in captive tapirs (Ramsay and Zainuddin, 1993; Cruz-Aldán et al., 2006). In the present study, we did not pursue further identification of the enteric ciliates; however, based on cytomorphology and previous reports, the genus *Balantidium* is the most likely in these cases. Additionally, we observed enteric/colonic nematodiasis in seven animals. In these cases, the nematodes had histomorphological features compatible with ascaridids. Several enteric nematode species have been reported in tapirs: *Parascaris* spp. *Paranoplocephala* spp., *Strongyloides* spp., *Agriostomun* spp., *Trichostrongylus* spp., *Strongylus* spp. And *Ascarididae* (Mangini, 2007; Zimmerman and Hernandez, 2014). Parasitological analysis revealed five species of helminths: *Cladorchis asper, C. pyriformis, K. longipene, M. monosticha* and *Physocephalus* spp.

In the tongue, we observed superficial glossitis (n = 9), mainly associated with eosinophilic infiltrates and occasional chronic proliferative endarteritis with fibromuscular intimal proliferation, stenosis of medium calibre, deep glossal arteries (n = 3) and granulomatous glossitis (n = 2). The aetiology of these lesions was not evident; nevertheless, main consideration was given to vascular nematodiasis as these lesions resemble those observed in elaeophoriasis (Couvillion et al., 1986) and

onchocerciasis (Williams et al., 1986), among others (Rice, 1995; Carpenter, 1998). *Sarcocystis* spp. infection, a common protozoan associated with glossitis (Robinson and Robinson, 2015), was not evident. Granulomatous glossitis in both cases was linked to plant material foreign bodies (Uzal et al., 2015).

Pulmonary anthracosis was observed in 12 tapirs. This is a common incidental finding in urban animals or those cohabiting with cigarette smokers (Caswell and Williams, 2015) and has been suggested as an indicator of air quality (Balchum et al., 1963). Studies in zoo animals have shown that anthracosis correlates with age and appears to be more prominent in longlived individuals (Ahasan et al., 2010). In the present study, we believe the main sources of anthracosis-related particles stemmed from: (1) burning of native forest, sugar cane plantations and eucalyptus for charcoal production; and (2) exhaust fumes from motor vehicles. Road-killed animals may be valuable bioindicators of environmental degradation and this observation has potential implications for wildlife and public health.

We observed eosinophilic and lymphoplasmacytic pericholangitis/cholangitis in nine tapirs; an aetiology was not readily evident in these cases. However, similar inflammatory infiltrates are primarily ascribed to parasitic infestation of the biliary tree by trematodes and nematodes in many vertebrates (Cullen and Stalker, 2015). Although we could not confirm the aetiology in these cases, *Ascaris* spp. And *Fasciola hepatica*, which have been previously reported in coproparasitological studies in lowland tapirs (Mangini et al., 2012), were considered reasonable possibilities.

Concerning the reproductive system, we observed lymphoplasmacytic interstitial orchitis/periorchitis in one male and chronic lymphoplasmacytic mastitis in two females. An aetiology for orchitis/periorchitis was not apparent. Similar lesions are often linked to brucellosis in various domestic species and wildlife (Foster, 2015; Terio et al., 2018). Previous studies evaluating anti-*Brucella* antibodies in wild tapirs yielded negative results in Costa Rica (Hernandez-Divers et al., 2005) and Brazil (Furtado et al., 2010; Medici et al., 2014). Serum samples from 35 tapirs captured in the same study area were tested for *Brucella abortus* and no antibodies were detected (LTCI, IPÉ, unpublished data). Porcine parvovirus can also cause reproductive pathology, and antibodies against this agent have already been detected in wild tapirs (Medici et

al., 2014). *Mycoplasma* spp. and lentiviruses, among others, are common pathogens involved in lymphoplasmacytic mastitis in cows, goats and sheep (Schlafer and Foster, 2015). The aetiology in these two cases remains unknown.

MVC-associated injuries were more prevalent in the TX and PA and less prevalent in the EX or in the HE. A literature review regarding MVC revealed skeletal fractures (e.g. skull, ribs) and brain contusion as the most common injuries in human MVCs (Toro et al., 2005; Yang, 2005; Burke, 2006), while long bone fractures are most frequent in small animals (Kolata and Johnston, 1975; Powell et al., 1999; Fighera et al., 2008; Streeter et al., 2009). Comparatively, little information is available on MVC injuries in large animals, namely cows and horses. We believe that this difference in traumatic injury distribution is associated with the anatomical characteristics of the species. Tapirs have short extremities, a robust thorax and abdomen and an adult body weight of up to 150e250 kg. Our results suggest that most tapirs suffer MVC primary impacts on the chest and abdomen. Interestingly, some animals had evidence of recent foraging, as indicated by abundant gastric ingesta. Post-prandial status in a subset of these animals could have played a role in diminished flight response capabilities.

Four animals presented with granulomatous lymphadenitis, lesions suggestive of paratuberculosis and other mycobacteriosis. However, ZN staining failed to detect acid alcohol-resistant bacteria. Other aetiologies such as parasites, bacteria or toxic plant exposure (e.g. *Vicia villosa*) could not be ruled out. From these, parasitic migration seems most plausible. Chronic tracheitis was observed in 11 tapirs. This lesion is likely of viral and/or bacterial nature (Caswell and Williams, 2015). In tapirs, there are serological reports of infectious bovine rhinotracheitis virus and herpesvirus infections (Mangini et al., 2012). However, we could not elucidate the aetiology of this lesion. Additionally, we observed gastric nodules associated with *Physocephalus* spp. Based on previous reports, consideration is given to *P. nitidulans* and *P. meridionalis*, which have been described in the stomach of lowland tapirs (Vicente et al., 1997).

Three animals presented with non-suppurative myocarditis, a lesion compatible with previous reports of encephalomyocarditis virus and neosporosis (Peters et al., 2017; Vercammen et al., 2017). In one animal, we observed lymphocytic meningitis, a

lesion usually observed in tapirs with neosporosis (Peters et al., 2017). However, we could not confirm the aetiology of these lesions. An ectopic intrahepatic haemopoietic nodule mainly comprised of CD3+ lymphocytes was seen in the liver of a fetus. To our knowledge, this is likely an incidental finding.

Ticks were prevalent in this set of tapirs, in agreement with previous observations (Mangini, 2007; Zimmerman and Hernandez, 2014). Tick infestations in tapirs are known to include the genera *Amblyomma* (18 species), *Ixodes* (seven species), *Haemaphysalis*, *Dermacentor*, *Rhipicephalus* and *Ornithodoros* (two species each) (Labruna and Guglielmone, 2009). All of the ticks observed in these animals were of the family Argasidae; however, no lesions were readily apparent at the attachment site. Few reports have associated the level of infestation with pathogenicity in the host (Quse and Fernandes-Santos, 2014). Future studies may aim at identifying the diversity of argasids in these tapirs.

In summary, through the systematic pathological study of road-killed tapirs, various novel pathological findings or disease processes were observed. Our results highlight the richness of wildlife health information obtained through the study of carcasses of road-kills. These cadavers can be useful to understand the health of different species in a regional and/or local environment. We recommend this type of study for other taxa where health information is scarce. The pathological information obtained through this study has revealed the diversity of infectious and noninfectious processes and/or aetiologies to which tapir populations in the Cerrado are exposed. Similar studies can be used in epidemiological surveillance programmes for livestock and zoonotic diseases as well as for environmental contamination. In addition, this study identified various novel pathological findings in free-ranging lowland tapirs. Although the aetiopathogenesis was not readily evident for some of them, parasitic infections appear to be prevalent. Moreover, we provide baseline pathology knowledge of lowland tapirs in South America, specifically in the Brazilian Cerrado, and our results may be helpful to road-kill first responders, caregivers, veterinarians and diagnosticians.

Conflict of Interest Statement

The authors declare no conflict of interest with respect to the publication of this manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2019.05.004.

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3.2 ANATOMOPATHOLOGIC CHARACTERIZATION OF TRAUMATIC INJURIES BY MOTOR-VEHICLE COLLISIONS IN NEOTROPICAL WILD MAMMALS

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3.2.1 **ABSTRACT**

Motor-vehicle collisions (MVC) involving wildlife are a severe threat to biodiversity worldwide, and current studies show that most vertebrate species are at risk. Although trauma by MVC is one of the major causes of death of wildlife, there is a considerable knowledge gap on traumatic features and potential patterns of MVC in wildlife. This study aimed to characterize MVC-traumatic injuries (MVC-TI) in neotropical wild mammals from Brazil. Injuries were classified topographically into four categories: abdomen/pelvis (AP), chest (TX), head/neck (HN), and extremities (EX). Additionally, we determined the prevalence of pathologic changes in MVC fatalities. A total of 430 wild mammals representing 44 different species were studied. AP (n=381; 89%) was the most affected body segment, followed by TX (n=372; 87%), HN (n=363; 84%) and EX (n=288; 67%). Most prevalent gross pathologic findings were single or multiple bone fractures (n=397; 92%), visceral rupture (n=371; 86%), haemothorax (n=220; 51%), and pulmonary haemorrhage (n=212; 49%). Microscopically, pulmonary oedema (n=324; 82%), and haemorrhage (n=272; 69%) were the most prevalent. These results enabled baseline delineation and characterization of MVC-TI in a large and diverse set of neotropical wild mammals. No distinct traumatic injury patterns were readily evident across the taxonomic groups; however, trend patterns were observed in some taxa, such as armadillos. These results may help clinicians performing emergency care in MVC patients and may be of value in pathologic and forensic investigations wherein MVC has been deemed a likely contributor factor of death.

Keywords: Blunt force trauma, Wildlife Vehicle Collisions, Pathology, Roadkill, Trauma, Wildlife.

3.2.2 **INTRODUCTION**

According to the World Health Organization (WHO), 1.3 million people die in traffic accidents annually, causing costs close to 3% of GDP in most countries (WHO, 2013). Therefore, understanding the biomechanics of traumatic injury (BTI) from Motor-vehicle collisions (MVC) is of medical relevance and should be a matter of public

health. For instance, analyses of MVC-traumatic injuries (MVC-TI) in pedestrians have resulted in the development and implementation of design modifications in vehicles seeking to reduce the severity of injuries (Eid & Abu-Zidan, 2007; Yang 2015). Also, understanding BTI has enabled understanding of MVC primary and secondary traumatic injuries, resulting in advanced emergency treatment protocols (Mackay, 1994; Ballesteros et al, 2004; Matsui, 2005; Strandroth et al, 2011). Unfortunately, comparatively, there are minimal reports on BTI in animals with few accounts in dogs and cats (Kolata et al, 1974; Marmarou et al, 1994; LaPlaca et al, 2007). Furthermore, the BTI states that the correlation between energy and mass is linear, while the correlation between energy and speed is exponential. Therefore, speed and vehicle size are fundamental for the extent and severity of injuries (Yang, 2005).

In wildlife, direct mortality from MVC is a leading threat and may drive local population decline (Brockie et al. 2009; Navas-Suárez et al, 2019). Studies have shown that essentially any vertebrate or invertebrate species is at risk (Rytwinski et al, 2016). Estimates are impressive; millions of vertebrates die on the world roads annually (Schwartz et al, 2020). Hotspots for MVC fatalities in wildlife are related to temporal and environmental characteristics, for example, road design, traffic, and surrounding landscape (Forman et al, 2003; Santos et al, 2017). However, there is very limited and fragmentary information describing the pathologic features of MVC in wildlife (Navas-Suarez et al, 2018; Garcês 2021). In order to partially fill in this knowledge gap, this study aimed to characterize and delineate the anatomopathologic features of MVC-TI in a large set of neotropical wild mammals from Brazil.

3.2.2 MATERIAL AND METHODS

The animals examined in this study comprised road-killed animals collected via a) toll road monitoring (TRM), b) active road monitoring (ARM), and c) passive collection (PC) of carcasses by public or private institutions. TRM was executed in partnership with four toll road companies. Each carcass was placed in biologic risk-plastic bags and stored frozen at operational control centres until transportation to the laboratory. ARM was implemented through a partnership with two nongovernmental

institutions (Wild Animal Conservation Institute -ICAS, and Lowland Tapir Conservation Initiative - INCAB) that monitored some highways in the state of Mato Grosso do Sul (MS). In this case, the data collection and necropsy were carried out in situ. PC involved partnership with entities that work with wild mammals since many made sporadic collections of MVC-injured animals; these were placed in biologic risk-plastic bags and stored frozen until transportation to the laboratory. Details of roads monitored are recorded in table 1. The partnership lasted 37 months (January 2017 through January 2020).

Table 1. General characteristics of the roads and type of carcass collection.

Institution	Type of collection	States	Road			
			Total km	Number	Type, material	Type, design
PI-01	ARM, PC	MS	1158	10	Paved	Mixed (two-lane, 2-1-lane, single-lane)
PI-02	ARM	MS	532	4	Paved	Mixed (two-lane, 2-1-lane, single-lane)
PI-03	TRM	SP	50	1	Paved	Two-lane
PI-04	TRM	SP, PR	402	1	Paved	Mixed (two-lane, single lane)
PI-05	TRM	SP	82	3	Paved	Mixed (two-lane, single lane)
PI-06	PC	SP	-	5	Paved	-
PI-07	PC	SP	-	2	Paved, unpaved	-
PI-08	PC	SC, PR	-	14	Paved (9), unpaved (5)	-

PI: partner institution; ARM: active road monitoring; TRM: toll road monitoring; PC: passive collection; MS: Mato Grosso do Sul State; SP: São Paulo State; PR: Paraná State; SC: Santa Catarina State.

Necropsy examinations followed a standard protocol and were performed only in carcasses deemed fresh or with mild or moderate autolysis (Erlandsson & Munro, 2007; King et al, 2014). The following data were collected for each carcass evaluated: date, GPS coordinates, sex (male, female), age class (juvenile/subadult, adult), and nutritional status (cachectic, moderate, or good). For taxonomic identification, we used the Brazilian mammal species identification guide (Reis et al, 2015). Additionally, for each species, specialized literature was reviewed to characterize the type of locomotion, activity pattern, diet, plasticity to land-use changes, and social habits (artiodactyls/perissodactyls (Wilson et al, 2011); carnivores (Shipping, 2009); didelphimorphids (Vernes, 2016); Primates (Mittermeier et al, 2013); and rodents

(Zima, 2017)). Supplemental table 1 details these characteristics for each species studied.

Traumatic injuries were classified based on the categories used by Kolata (Kolata et al, 1974), namely, head and neck (HN), thorax (TX), abdomen/pelvis (AP), and extremities (EX). Furthermore, blunt force injuries were described and recorded based on terminology recommended by Ressel (Ressel et al, 2016). Representative tissue samples were collected and fixed in 10% neutral buffered formalin. Tissue samples were processed routinely and embedded in paraffin-wax. For histologic examination, sections (5 μm) were stained with haematoxylin and eosin (HE). Supplemental Table 2 details the organs/tissues evaluated. Microscopic findings were classified into MVC-TI and non-MVC-TI. In addition, the microscopic findings were categorized according to specific literature (Shkrum & Ramsay, 2007).

To better understand bone trauma, we performed full-body radiologic evaluations of 40 individuals being 14 Carnivora, eight Pilosa, six Rodentia, five Didelphimorphia, four Cingulata, two Primates, and one Lagomorpha. Radiographs included two projections, lateral (L) and ventrodorsal (VD), and two equipment sets were used, a 500 mA X-ray machine (Tecno Designer®) coupled with a computed radiography system (CR, Fuji®) and a 625 mA X-ray machine (Agfa®) with a digital radiography system. For data evaluation, descriptive data analysis was performed. Data were reported in absolute (n = number of cases) and relative (%) terms.

The Ethics Committee on Animal Use on Research of the FMVZ/USP approved this study under protocol number: 7198020317, and the System Authorization and Information on Biodiversity (SISBIO) of the Chico Mendes Institute of Biodiversity Conservation (ICMBio) approved this study with the license number: 27587-12.

3.2.3 **RESULTS**

Study area, epidemiology, and biologic characteristics of investigated animals

A total of 430 wild mammals, including 44 species, 21 families, and 9 orders, were analyzed (Figure 1). In general, the five most prevalent species were: giant

anteater (Myrmecophaga tridactyla, n=61), crab-eating fox (Cerdocyon thous, n=44), southern tamandua (Tamandua tetradactyla, n=40), Brazilian tapir (Tapirus terrestris, n=36), and nine-banded armadillo (Dasypus novemcinctus, n=24). Overall, taxonomic order distribution was: Carnivora (n=138; 32%), Pilosa (n=106; 25%), Cingulata (n=50; 12%), Perissodactyla (n=36; 8%), Rodentia (n=35; 8%), Artiodactyla (n=21; 5%), Didelphimorphia (n=21; 5%), Primates (n=20; 5%), and Lagomorpha (n=3; 1%). Distribution of ecologic, spatial, and biologic characteristics are recorded in Figure 2.

Figure 1. Spatial distribution of Brazilian wild mammals included in this study.
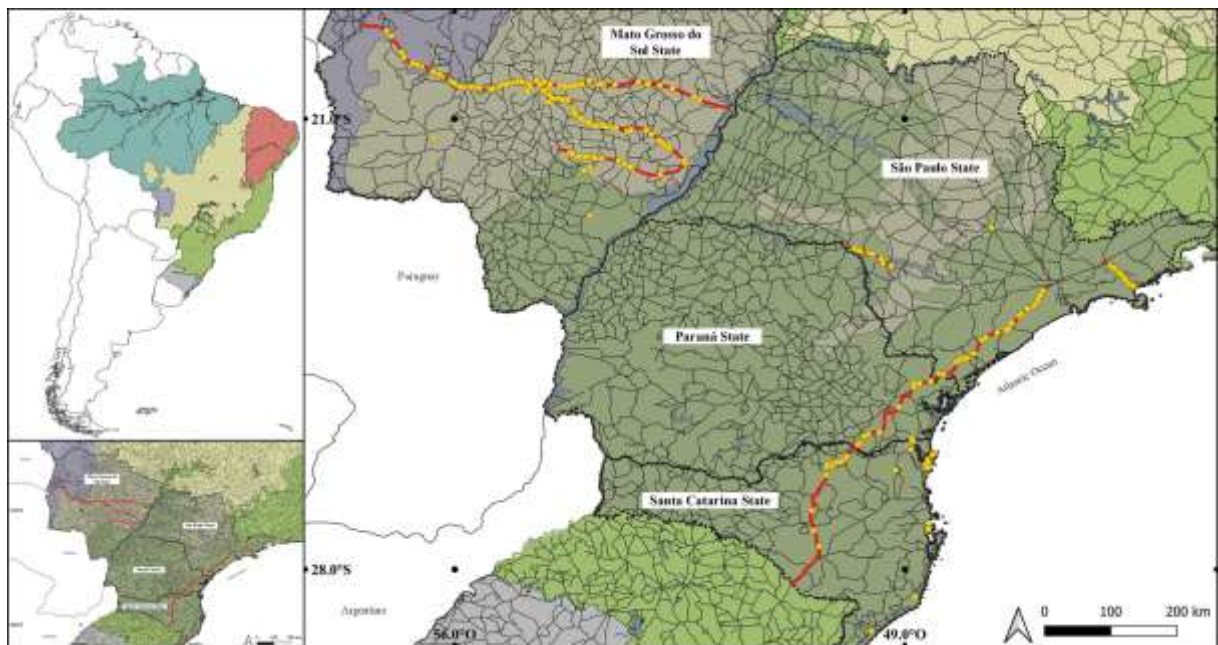
Topographic classification of MVC-trauma and biologic associations

In decreasing order, MVC-TI distribution was AP (n=381; 89%), TX (n=372; 86%), HN (n=363; 84%), and EX (n=288; 67%). Characteristics or parameters with a frequency greater than 90% per anatomic distribution were as follows. In AP, order (Didelphimorphia, Perissodactyla, and Rodentia), locomotion type (scansorial, semiaquatic, and terrestrial), activity pattern (diurnal/nocturnal, nocturnal), diet (insectivore), biome (Cerrado), body-size (large, small). In TX, Order (Carnivora, Perissodactyla, Rodentia), locomotion type (semiaquatic), and social habits (small

groups). In HN, Order (Carnivora, Rodentia), locomotion type (arboreal, semiaquatic), diet (carnivore, herbivore), social habits (gregary, small groups), age class (infant/juvenile), body-size (small). Moreover, for EX, none of the characteristics or parameters had a frequency greater than 90%. The frequency of the ecologic/spatial/biologic variables concerning the MVC-TI topography is illustrated in Figure 3.

Figure 2. Ecologic, spatio-temporal, and biologic characteristics of Brazilian wild mammals killed by motor vehicle collision (MVC).
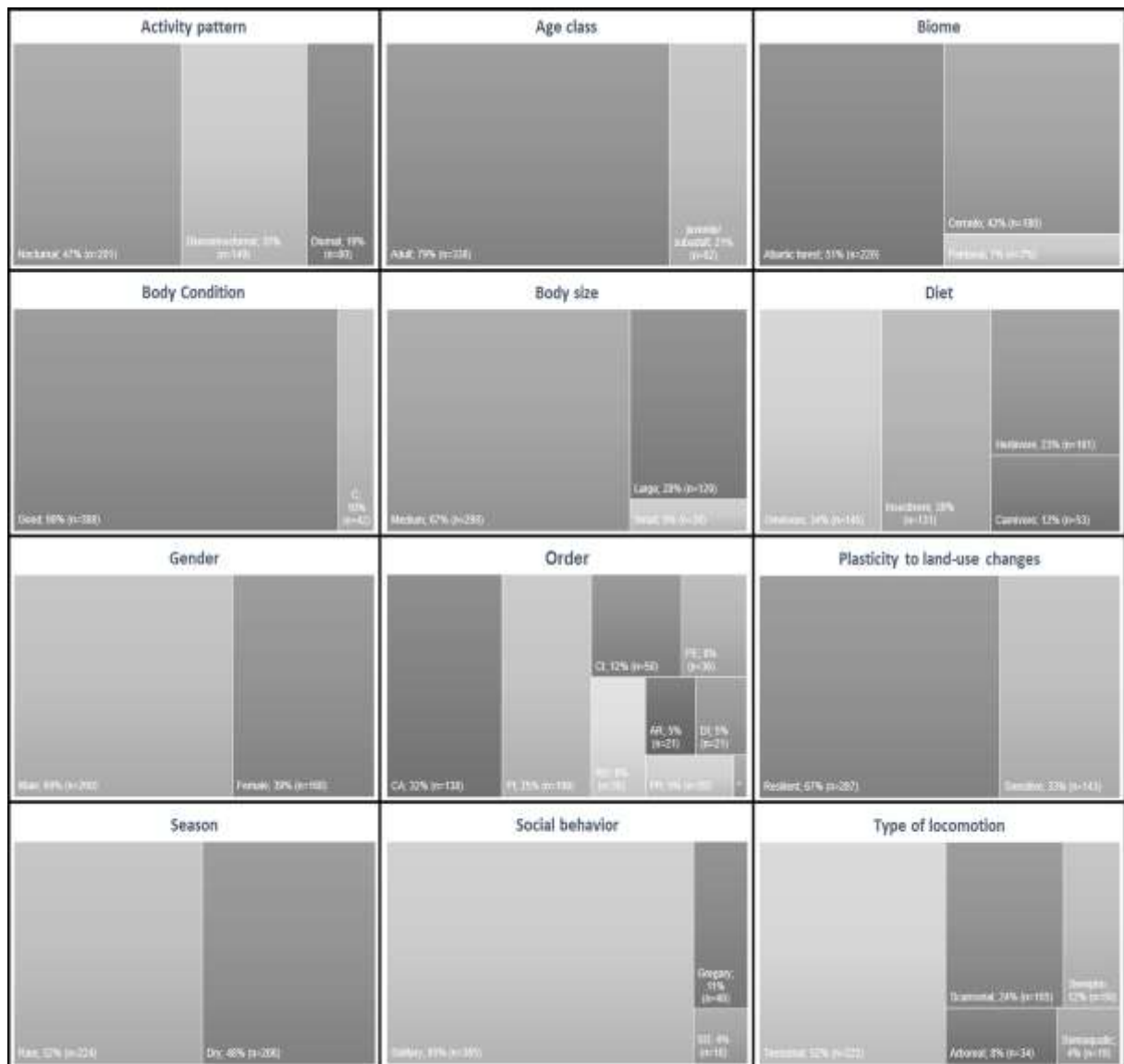
Figure 3. Ecologic, temporal, and biologic characteristics in relation to topographic sections affected by MVC-traumatic injuries in Brazilian wild mammals.
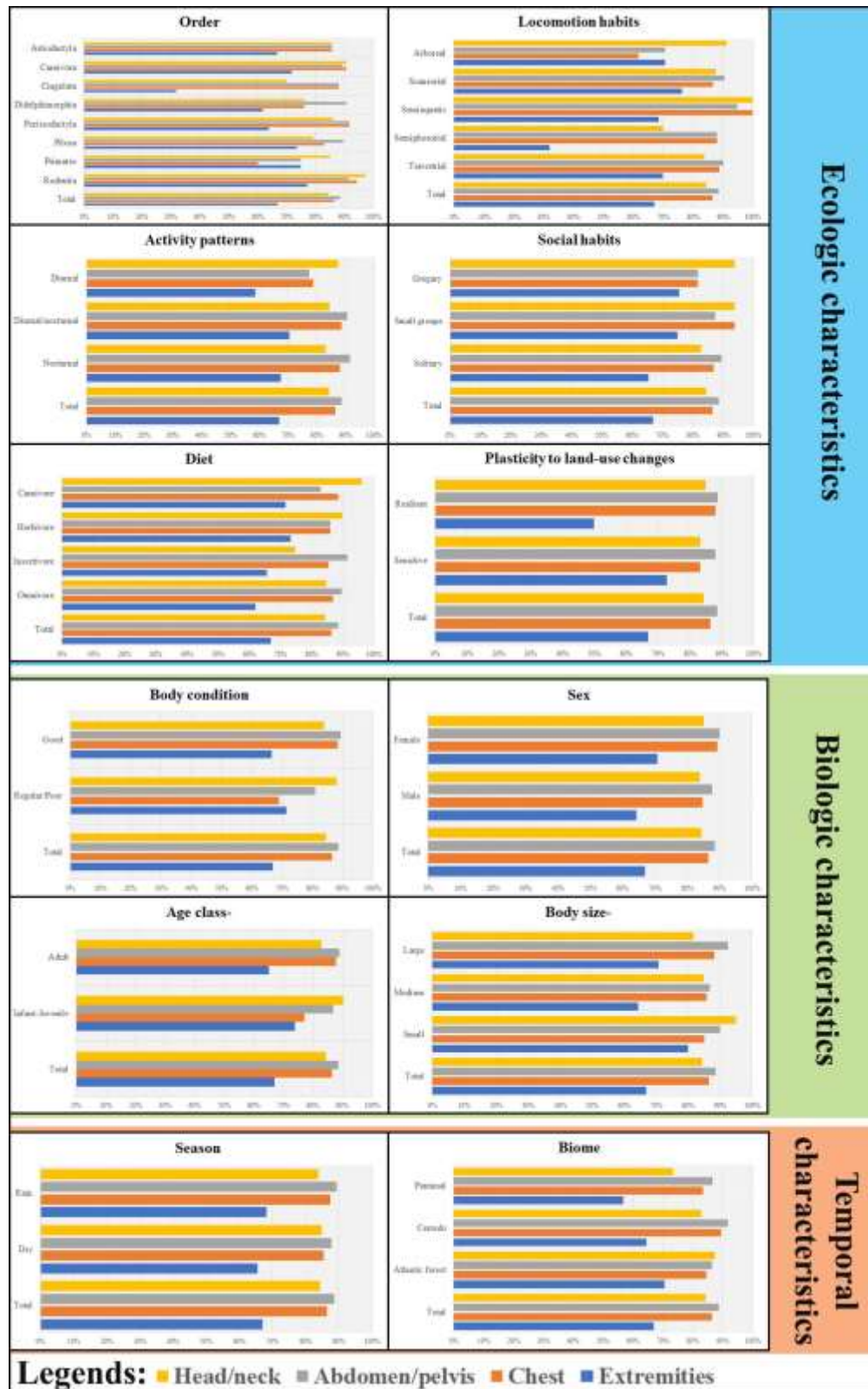
According to the number of segments affected, 207 (48%) animals had all four segments, 148 (34%) three segments, 59 (14%) two segments, and 14 (3%) only one segment compromised. Rodents had a frequency of lesions greater than 60% in all segments, whereas cingulata had 20%. Figure 4 depicts concurrent traumas and the relationship with the height of the bumper (HB) in cars and trucks.

Figure 4. Frequency of MVC-traumatic injuries by topographic region and concurrent segments in Brazilian wild mammals, as well as its relationship with the height of the bumper (HB), in cars (yellow), and trucks (red).
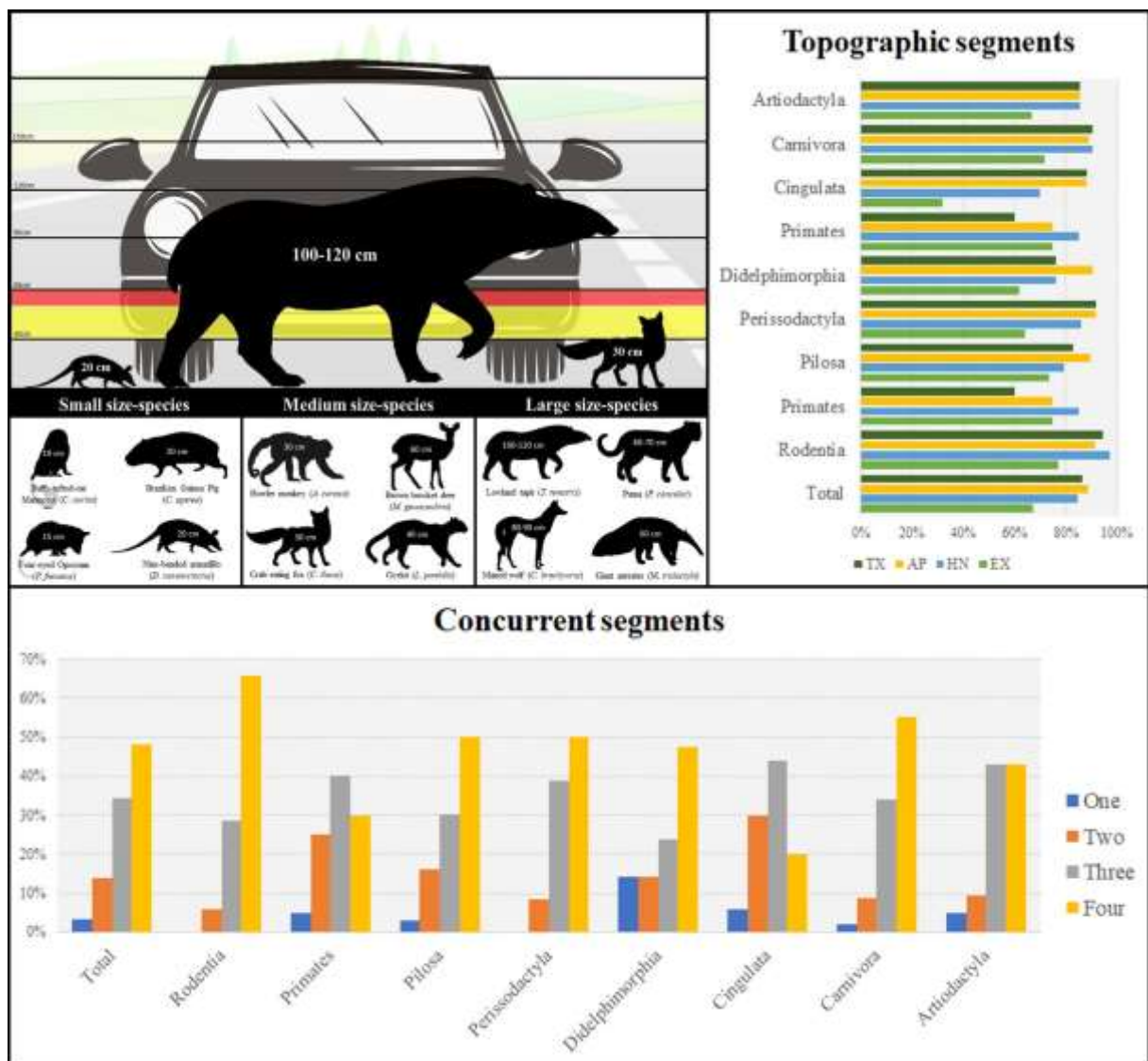
Pathologic findings

Gross pathologic findings with a frequency greater than 30% are recorded in Table 2. Most prevalent gross pathologic findings were single or multiple bone fractures (n=397; 92%), visceral rupture (n=371; 86%), haemothorax (n=220; 51%), pulmonary haemorrhage (n=212; 49%), cutaneous/subcutaneous contusions (n=210; 49%) and lacerations (n=207; 48%), and hemoperitoneum (n=169; 39%). Dilated stomach filled with ingesta was a frequent finding in Carnivora (n=83; 60%), Pilosa (n=61; 56%), Cingulata (n=25; 50%), Perissodactyla (n=18; 50%), Rodentia (n=19; 54%), Artiodactyla (n=13; 62%), Didelphimorphia (n=7; 33%), and Primates (n=6; 30%). Figure 5 illustrates main gross MVC-TI findings. Supplemental figures 1-5 illustrate MVC-TI gross findings for each taxonomic group.

In HN region, the most frequent findings were fracture of skull (n=236, 55%), including the nasal bones (n=127, 30%), haemorrhage in the oral cavity (n=130; 30%), and epistaxis (n=116; 27%). In TX region, haemothorax was the most prevalent gross finding (n=220; 51%), followed by pulmonary haemorrhage (n=212; 49%), and fracture of ribs (n=198; 46%). In AP region, most prevalent findings were liver rupture (n=251; 58%), fracture of hip (n=214; 50%), and hemoperitoneum (n=169; 39%). In the EX-region, there were no findings with an occurrence greater than 30%. The gross and microscopic (Table 3) MVC-TI findings with occurrence upper than 30% for each order included are described in the following paragraphs. Figure 6 illustrates the main microscopic MVC-TI findings.

Pathologic findings by Order

Carnivora

HN injuries (n=125; 91%) were characterized by craniofacial polytraumatism with fracture of temporal (n=87; 63%), occipital (n=81; 59%), parietal (n=76; 55%), mandibular (n=66; 48%), maxillary (n=59; 43%), frontal (n=58; 42%), and nasal (58; 42%) bones. TX injuries (n=126%) were haemothorax (n=75; 54%), fracture of ribs (n=67, 49%), pulmonary haemorrhage (n=59; 43%), and rupture of heart (n=43; 31%). In AP (n=123; 89%), there was common rupture of liver (n=85; 62%), hip fracture

(n=74; 54%), hemoperitoneum (52, 38%), and rupture of kidney (n=47; 34%) and spleen (n=42; 30%). In EX (n=99; 72%), there were no findings with an occurrence greater than 30%. Microscopically, main findings were pulmonary oedema (n=111; 89%), pulmonary haemorrhage (n=86; 69%), and acute segmental myofiber degeneration (n=18; 35%).

Figure 5. Main gross findings of motor vehicle collision-traumatic injuries (MVC-TI) in wild mammals.
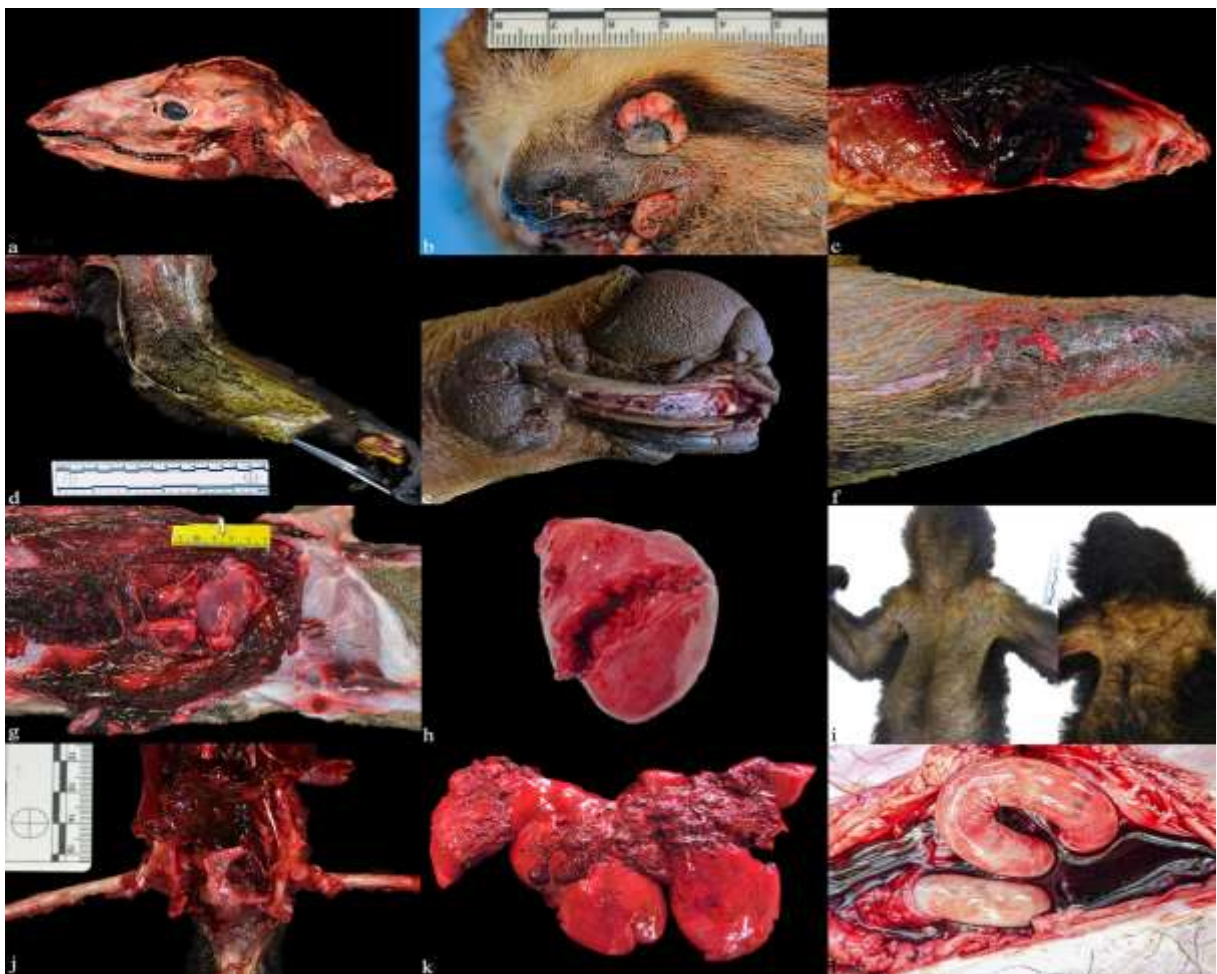
A. *Mazama gouazoubira*. Multiple fractures of temporal and parietal bones with rupture and hemorrhage of brain. **B.** *Bradipus variegatus*. Craniofacial polytraumatism with exophthalmia of left eye. **C.** *Galictis cuja*. Marked subcutaneous hemorrhage along the cranial region. **D.** A*louatta caraya*. Presence of ingesta in the subcutis of the extremities. **E.** *Tamandua tetradactyla*. Rupture of claw. **F.** *Hydrochoerus hydrochaeris*. Cutaneous abrasion with hemorrhage in right foreleg. **G.** *Puma jagouaroundii*. Eventration of heart and pulmonary lobes through the subcutaneous axillary region and hemorrhage. **H**. *Cerdocyon thous*. Myocardial rupture along the interventricular septum. **I.** *Alouatta caraya*. In the left image pectoral region with vehicle paint debris and wheel marks, right image normal. **J.** *Callithrix aurita*. Subcutaneous hematoma in inguinal region and multiple fracture of pelvis with hemorrhage. **K**. *Puma concolor*. Rupture of hepatic parenchyma. **L.** *Myrmecophaga tridactyla*. Hemoperitoneum.

Table 2. Main gross pathologic findings in Brazilian wild mammals with motor vehicle collision-traumatic injuries (MVC-TI).

Gross findings	T	CA	PI	CI	PE	RO	AR	DI	PR
Fracture	397 (92%)	130 (94%)	92 (85%)	49 (98%)	31 (86%)	34 (97%)	19 (90%)	20 (95%)	19 (95%)
Skull, temporal	215 (50%)	87 (63%)	46 (43%)	18 (36%)	6 (17%)	23 (66%)	4 (19%)	14 (67%)	14 (70%)
Hip	214 (50%)	74 (54%)	50 (46%)	24 (48%)	14 (39%)	25 (71%)	5 (24%)	13 (62%)	8 (40%)
Skull, occipital	212 (49%)	81 (59%)	50 (46%)	18 (36%)	4 (11%)	24 (69%)	5 (24%)	14 (67%)	13 (65%)
Skull, parietal	200 (47%)	76 (55%)	48 (44%)	14 (28%)	6 (17%)	22 (63%)	6 (29%)	14 (67%)	11 (55%)
Rib	198 (46%)	67 (49%)	39 (36%)	19 (38%)	20 (56%)	21 (60%)	10 (48%)	11 (52%)	9 (45%)
Jaw	146 (34%)	66 (48%)	13 (12%)	12 (24%)	11 (31%)	16 (46%)	4 (19%)	13 (62%)	9 (45%)
Skull, frontal	139 (32%)	58 (42%)	28 (26%)	10 (20%)	3 (8%)	13 (37%)	6 (29%)	13 (62%)	5 (25%)
Nasal	127 (30%)	58 (42%)	23 (21%)	7 (14%)	3 (8%)	18 (51%)	4 (19%)	7 (33%)	6 (30%)
Maxillae	121 (28%)	59 (43%)	13 (12%)	7 (14%)	4 (11%)	14 (40%)	2 (10%)	12 (57%)	9 (45%)
Femur	105 (24%)	37 (27%)	28 (26%)	12 (24%)	11 (31%)	12 (34%)	1 (5%)	1 (5%)	3 (15%)
Rupture	371 (86%)	118 (86%)	95 (88%)	43 (86%)	34 (94%)	29 (83%)	17 (81%)	17 (81%)	15 (75%)
Liver	251 (58%)	85 (62%)	75 (69%)	25 (50%)	17 (47%)	19 (54%)	9 (43%)	13 (62%)	7 (35%)
Kidney	131 (30%)	47 (34%)	44 (41%)	13 (26%)	7 (19%)	12 (34%)	2 (10%)	4 (19%)	1 (5%)
Spleen	131 (30%)	42 (30%)	43 (40%)	10 (20%)	22 (61%)	8 (23%)	2 (10%)	0 (0%)	3 (15%)
Lungs	107 (25%)	38 (28%)	21 (19%)	6 (12%)	18 (50%)	11 (31%)	7 (33%)	2 (10%)	2 (10%)
Heart	106 (25%)	43 (31%)	25 (23%)	11 (22%)	9 (25%)	9 (26%)	2 (10%)	4 (19%)	2 (10%)
Diaphragm	99 (23%)	38 (28%)	26 (24%)	4 (8%)	12 (33%)	7 (20%)	2 (10%)	4 (19%)	5 (25%)
Hemorrhage	306 (71%)	92 (67%)	83 (77%)	35 (70%)	30 (83%)	20 (57%)	17 (81%)	12 (57%)	15 (75%)
Lungs	212 (49%)	59 (43%)	56 (52%)	23 (46%)	28 (78%)	14 (40%)	14 (67%)	7 (33%)	9 (45%)
Oral cavity	130 (30%)	32 (23%)	40 (37%)	12 (24%)	11 (31%)	11 (31%)	10 (48%)	8 (38%)	4 (20%)
Nostrils	116 (27%)	31 (22%)	29 (27%)	13 (26%)	12 (33%)	10 (29%)	8 (38%)	8 (38%)	3 (15%)
Hemothorax	220 (51%)	75 (54%)	45 (42%)	30 (60%)	27 (75%)	17 (49%)	11 (52%)	9 (43%)	5 (25%)
Cutaneous/subcutaneous contusion	210 (49%)	76 (55%)	51 (47%)	11 (22%)	16 (44%)	16 (46%)	16 (76%)	9 (43%)	13 (65%)
Skin laceration	207 (48%)	65 (47%)	41 (38%)	37 (74%)	17 (47%)	15 (43%)	13 (62%)	3 (14%)	13 (65%)
Hemoperitoneum	169 (39%)	52 (38%)	47 (44%)	13 (26%)	32 (89%)	11 (31%)	6 (29%)	5 (24%)	3 (15%)
Pulmonary edema	122 (28%)	29 (21%)	34 (31%)	19 (38%)	19 (53%)	4 (11%)	6 (29%)	4 (19%)	6 (30%)
Skin abrasion	90 (21%)	18 (13%)	23 (21%)	4 (8%)	27 (75%)	7 (20%)	7 (33%)	2 (10%)	2 (10%)
Stomach full of feed	234 (54%)	83 (60%)	61 (56%)	25 (50%)	18 (50%)	19 (54%)	13 (62%)	7 (33%)	6 (30%)

T=total; CA=Carnivora; PI=Pilosa; CI=Cingulata; PE=Perissodactyla; RO=Rodentia; AR=Artiodactyla; DI=Didelphimorphia; PR=Primates.

Table 3. Main histopathologic findings in Brazilian wild mammals with motor vehicle collision-traumatic injuries (MVC-TI).

MVC-TI microscopic findings	T	CA	PI	CI	PE	RO	AR	DI	PR
Pulmonary edema	324 (82%)	111 (89%)	88 (89%)	39 (83%)	9 (26%)	25 (89%)	20 (95%)	12 (67%)	17 (89%)
Pulmonary hemorrhage	272 (69%)	86 (69%)	76 (77%)	39 (83%)	19 (56%)	17 (61%)	14 (67%)	7 (39%)	12 (63%)
Adrenal gland hemorrhage	47 (28%)	8 (17%)	29 (50%)	4 (33%)	2 (13%)	1 (9%)	1 (10%)	0 (0%)	2 (20%)
Cerebral hemorrhage	35 (25%)	7 (22%)	15 (32%)	3 (50%)	7 (27%)	0 (0%)	1 (10%)	0 (0%)	2 (25%)
Myofiber degeneration	43 (25%)	18 (35%)	14 (30%)	3 (19%)	2 (18%)	3 (21%)	1 (6%)	0 (0%)	2 (22%)
Pulmonary congestion	86 (22%)	24 (19%)	22 (22%)	28 (60%)	5 (15%)	4 (14%)	0 (0%)	2 (11%)	0 (0%)
Hemorrhage of spleen	60 (21%)	11 (11%)	21 (27%)	5 (23%)	17 (57%)	2 (10%)	3 (16%)	1 (11%)	0 (0%)
Myofiber rupture	36 (21%)	14 (27%)	10 (22%)	2 (13%)	0 (0%)	5 (36%)	2 (13%)	1 (13%)	2 (22%)
Cerebellar hemorrhage	10 (18%)	2 (15%)	8 (26%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hyperinsuflation	58 (15%)	14 (11%)	24 (24%)	0 (0%)	11 (32%)	2 (7%)	3 (14%)	2 (11%)	2 (11%)
Pancreatic hemorrhage	15 (13%)	0 (0%)	11 (19%)	4 (44%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Alveolar bullae	49 (12%)	18 (14%)	23 (23%)	0 (0%)	1 (3%)	1 (4%)	4 (19%)	1 (6%)	1 (5%)
Esophageal hemorrhage	23 (10%)	3 (4%)	16 (24%)	2 (13%)	1 (8%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)
Hemorrhage of lymph node	14 (10%)	1 (2%)	5 (13%)	0 (0%)	8 (40%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Renal hemorrhage	33 (10%)	9 (8%)	12 (13%)	1 (4%)	9 (29%)	2 (7%)	0 (0%)	0 (0%)	0 (0%)
Hepatic hemorrhage	29 (9%)	5 (5%)	14 (15%)	3 (10%)	6 (19%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)
Renal proteinosis	29 (9%)	3 (3%)	18 (20%)	4 (14%)	2 (6%)	1 (4%)	1 (5%)	0 (0%)	0 (0%)
Tracheal hemorrhage	26 (9%)	4 (4%)	18 (24%)	2 (10%)	1 (4%)	0 (0%)	0 (0%)	1 (7%)	0 (0%)
Alveolar rupture	27 (7%)	4 (3%)	13 (13%)	0 (0%)	2 (6%)	1 (4%)	6 (29%)	0 (0%)	1 (5%)
Hepatic congestion	23 (7%)	2 (2%)	18 (19%)	1 (3%)	2 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Myocardial hemorrhage	15 (4%)	4 (4%)	7 (8%)	1 (3%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	2 (11%)
Splenic congestion	11 (4%)	0 (0%)	2 (3%)	0 (0%)	9 (30%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tubular degeneration, kidney	12 (4%)	1 (1%)	4 (4%)	0 (0%)	6 (19%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cardiomyocyte degeneration	11 (3%)	2 (2%)	5 (6%)	0 (0%)	3 (9%)	0 (0%)	0 (0%)	1 (7%)	0 (0%)
Pulmonary embolism	13 (3%)	4 (3%)	8 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)

T=total; CA=Carnivora; PI=Pilosa; CI=Cingulata; PE=Perissodactyla; RO=Rodentia; AR=Artiodactyla; DI=Didelphimorphia; PR=Primates

Figure 6. Microscopic findings associated with MVC-traumatic injury.
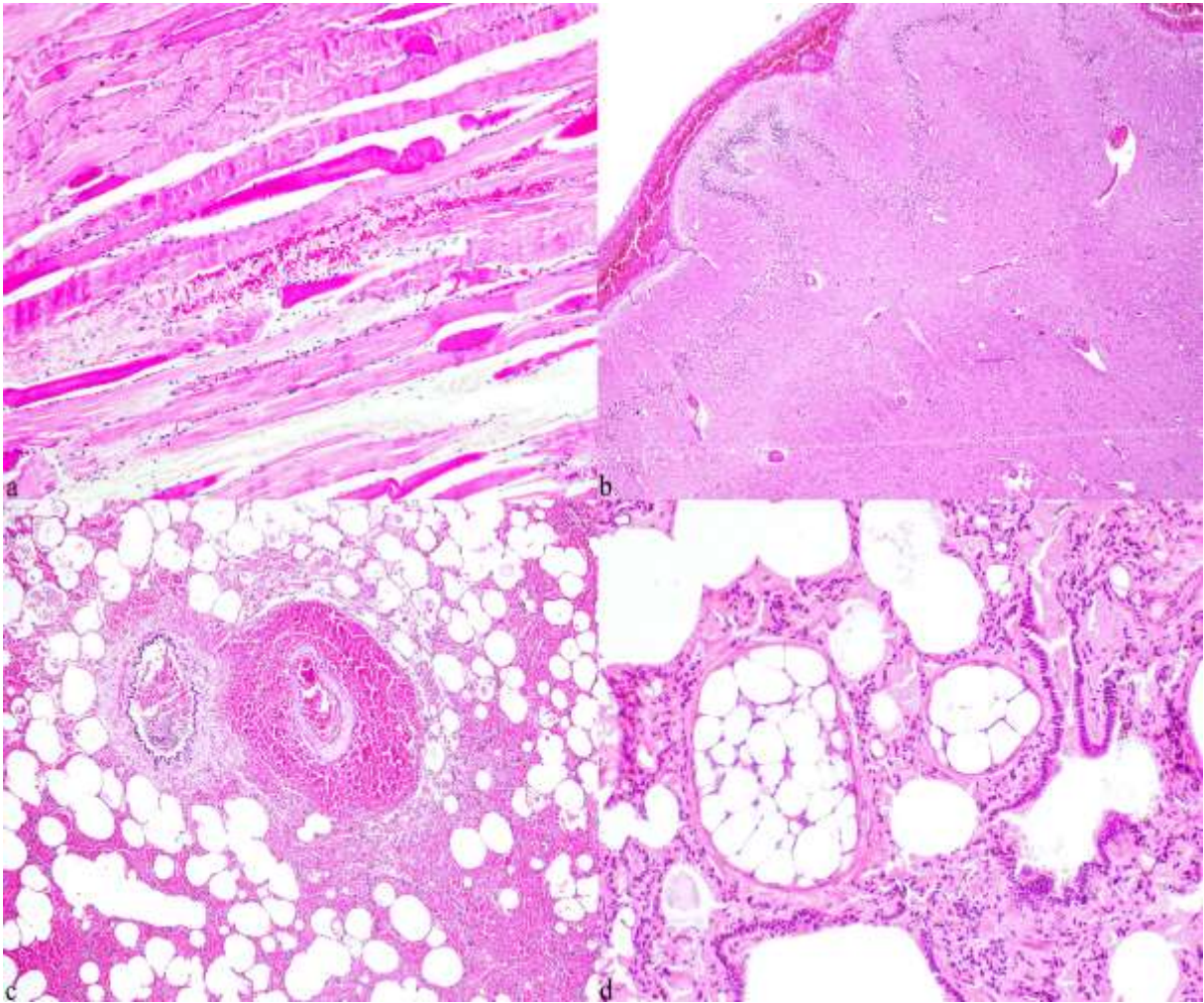
A. *Cerdocyon thous*. Skeletal muscle. Acute degenerative changes including hypereosinophilic, contracted (arrow) and fragmented myofibers (arrowheads) and interstitial hemorrhage. HE 100X. **B**. *Myrmecophaga tridactyla*. Central nervous system. Acute subarachnoid/leptomeningeal hemorrhage (black arrow). HE 40X. **C**. *Cerdocyon thous*. Lungs. Acute perivascular hemorrhage (arrow). HE 40X. **D**. *Tamandua tetradactyla*. Lungs. Bone narrow emboli (asterisks). HE 200X.

Pilosa

In anteaters and sloths, AP (n=95; 90%) injuries were the most frequent and included rupture of liver (n=75; 69%), fracture of hip (n=50; 46%), hemoperitoneum (n=47; 44%), and rupture of kidney (n=44; 41%) and spleen (n=43; 40%). In TX (n=88; 83%), pulmonary haemorrhage (n=56; 52%), haemothorax (n=45; 42%), fracture of ribs (n=39; 36%), and pulmonary oedema (n=34; 31%) were common. In HN (n=84;

79%), fracture of occipital (n=50; 46%), parietal (n=48; 44%) and temporal (n=46; 43%) bones, as well as haemorrhage in the oral cavity (n=40; 37%) were most prevalent. In EX (n=78; 74%), there were no findings with an occurrence greater than 30%. Microscopically, main findings were pulmonary oedema (n=88; 89%), pulmonary haemorrhage (n=76; 77%), adrenal gland haemorrhage (n=29; 50%), cerebral haemorrhage (n=15; 32%), and acute segmental myofiber degeneration (n=14; 30%).

Cingulata

In armadillos, TX (n=44; 88%) and AP (n=44; 88%) were the most affected anatomic segments; main macroscopic findings were haemothorax (n=30; 60%), pulmonary haemorrhage (n=23; 46%), fracture of ribs (n=19; 38%), pulmonary oedema (n=19; 38%), fracture of thoracic vertebrae (n=16; 32%), abdominal skin lacerations (n=27; 54%), rupture of liver (n=25; 50%), fracture of hip (n=24; 48%) and lumbar vertebrae (n=18; 36%), and ventro-abdominal evisceration (n=18; 36%). Most common findings in HN (n=35; 70%) were fracture of temporal (n=18; 36%) and occipital (n=18; 36%) bones. In EX (n=16; 32%), there were no findings with an occurrence greater than 30%. Microscopically, main findings were pulmonary oedema (n=39; 83%), haemorrhage (n=39; 83%) and congestion (n=28; 60%), cerebral haemorrhage (n=3; 50%), pancreatic haemorrhage (n=4; 44%), and adrenal gland haemorrhage (n=4; 33%).

Perissodactyla

In tapirs, AP (n=33; 92%) and TX (n=33; 92%) were the most affected anatomic segments. Most prevalent macroscopic findings in AP were hemoperitoneum (n=32; 89%), rupture of spleen (n=22; 61%), stomach (n=18; 50%), liver (n=17; 47%), abdominal skin abrasion (n=17; 47%), fracture of hip (n=14; 39%), and rupture of diaphragm (n=12; 33%). In TX, pulmonary haemorrhage (n=28; 78%), haemothorax (n=27; 75%), thoracic skin abrasions (n=24; 67%), fracture of rib (n=20; 56%), pulmonary oedema (n=19; 53%), and rupture of lungs (n=18; 50%) prevailed. In HN (n=31; 86%), facial cutaneous abrasions (n=19; 53%), epistaxis (n=12; 33%), fracture

of mandible (n=11; 31%), and haemorrhage in the oral cavity (n=11; 31%) were most common. In EX (n=23; 64%), most prevalent finding was fracture of femur (n=11; 31%). Microscopically, main findings were splenic haemorrhage (n=17; 57%), pulmonary haemorrhage (n=19; 56%), haemorrhage of lymph nodes (n=8; 40%), alveolar emphysema (n=11; 32%), and splenic congestion (n=9; 30%).

Rodentia

In rodents, HN (n=34; 97) injury was most frequent. The main findings in this anatomic segment were fracture of occipital (n=24; 69%), temporal (n=23; 66%), parietal (n=22; 63%), nasal (n=18; 51%), mandibular (n=16; 46%), maxillary (n=14; 40%) and frontal (n=13; 37%) bones, and haemorrhage in the oral cavity (n=11; 31%). In TX (n=33; 94%), fracture of ribs (n=21; 60%), haemothorax (n=17; 49%), pulmonary haemorrhage (n=14; 40%), and rupture of the lungs (n=11; 31%) were most prevalent. In AP (n=32; 91), fracture of hip (n=25; 71%), rupture of liver (n=19; 54%), kidney (n=12; 34%), small intestine (n=12; 34%), large intestine (n=11; 31%), and hemoperitoneum (n=11; 31%) prevailed. In EX (n=27, 77%), fracture of femur and tibia (n=12; 34%, each) were common. Microscopically, main findings were pulmonary oedema (n=25; 89%), pulmonary haemorrhage (n=17; 61%), and acute segmental myofiber degeneration (n=5; 36%).

Artiodactyla

In deer, MVC-TI in anatomic segments HN, TX, and AP had identical occurrence (n=18; 86%). The main gross findings were, pulmonary haemorrhage (n=14; 67%), haemothorax (n=11; 52%), fracture of ribs (n=10; 48%), haemorrhage in the oral cavity (n=10; 48%) and in the ear (n=10; 48%), rupture of the liver (n=9; 43%), cutaneous/subcutaneous contusions in thorax (n=9; 43%), epistaxis (n=8; 38%), and rupture of the lungs (n=7; 33%). In EX (n=14; 67%), cutaneous laceration in forelegs (n=7; 33%) was common. Microscopically, main findings were pulmonary oedema (n=20; 95%) and haemorrhage (n=14; 67%).

Didelphimorphia

In opossums, AP (n=19; 90%) was the most affected anatomic segment and the main gross findings were fracture of hip (n=13; 62%) and rupture of liver (n=13; 62%). In HN (n=16; 76%), fracture of temporal (n=14; 67%), occipital (n=14, 67%), parietal (n=14; 67%), mandibular (n=13; 62%), frontal (n=13; 62%) and maxillary (n=12; 57%) bones, haemorrhage in the oral cavity (n=8, 38%), epistaxis (n=8, 38%), and fracture of nasal (n=7; 33%). In TX (n=16; 76), fracture of the ribs (n=11; 52%), haemothorax (n=9; 43%), and pulmonary haemorrhage (n=7; 33%) were most common. In EX (n=13; 62%), there were no findings with an occurrence greater than 30%. Microscopically, main findings were pulmonary oedema (n=12; 67%) and haemorrhage (n=7; 39%).

Primates

In New World primates, HN (n=17; 85%) was the most affected anatomic segment, and main gross findings were fracture of temporal (n=14; 70%), occipital (n=13; 65%), parietal (n=11; 55%), mandibular (n=9; 45%), maxillary (n=9; 45%) and nasal (n=6; 30%) bones. In AP (n=15; 75%), fracture of hip (n=8; 40%) and rupture of liver (n=7; 35%) were common. In EX (n=15; 75%), cutaneous lacerations in forelegs (n=9; 45%), fracture of radius (n=6; 30%), and luxation of elbow (n=6; 30%) were prevalent. In TX (n=15; 75%), fracture of ribs (n=9; 45%), pulmonary haemorrhage (n=9; 45%) and oedema (n=6; 30%) were frequent. Microscopically, main findings were pulmonary oedema (n=17; 89%), and haemorrhage (n=12; 63%).

Radiographic study

The main radiologic findings were fractures of hip (n=20; 50%), ribs (n=18; 45%), and frontal (n=14 ;35%), occipital (n=13; 32%) and temporal (n=12; 30%) bones. Miscellaneous findings were consolidated fractures (n=4; 40%), pregnancy (n=1; 1%), hemivertebra (n=1; 1%), supernumerary ribs (n=1; 1%), and presence of radiopaque structures consistent with lead projectiles in one P. yagouaroundi (n=1; 1%) that were confirmed at necropsy. Additionally, incompletely closed physes (n=22; 55%),

indicating osteologically immature animals, were seen in cases where the body mass suggested adult size. Table 4 records all radiologic findings. Hip fractures involved ilium and ischium in three cases (8%) each, pubic bone in one case (3%), and involved more than one bone in 14 cases (35%). Rib fractures were complete in 12 cases (30%), multiple in one case (3%); in 15 animals (38%), fractures involved more than one rib. Frontal bone fractures were multiple in 11 cases (28%). Figure 7 illustrates main radiographic MVC-TI findings.

Figure 7. X-ray/gross findings of motor vehicle collision-traumatic injuries (MVC-TI) in wild mammals.

A. *Puma concolor*. Multiple fractures in the nasal, frontal, and parietal bones. Note sinking of the nasal region and complete maxillary fracture. **B**. *Puma yagouaroundii*. Note the soft tissue radiopacity structure in the axillary region, eventrated heart (white arrow), rib fracture, and four round metal radiopacity structures compatible with lead projectiles (white arrowhead). Extensive subcutaneous hemorrhage. **C**. *Procyon cancrivorus*. Fracture-luxation of the first and second lumbar vertebra (circle and arrow). **D**. *Callithrix aurita*. Hip fracture (arrow) involving the pubis and ischium. Hematoma in the inguinal region, in the upper left image note fracture of the hip.

Table 4. Main radiologic findings in Brazilian wild mammals with motor vehicle collision-traumatic injuries (MVC-TI).

Radiologic findings	T	CA	PI	RO	DI	CI	PR	LA
Open epiphyseal plates	22 (55%)	8 (57%)	4 (50%)	2 (33%)	3 (60%)	3 (75%)	1 (50%)	1 (100%)
Fracture of hip	20 (50%)	5 (36%)	5 (63%)	3 (50%)	4 (80%)	1 (25%)	2 (100%)	0 (0%)
Fracture of ribs	18 (45%)	7 (50%)	3 (38%)	1 (17%)	4 (80%)	1 (25%)	1 (50%)	1 (100%)
Fracture of frontal bone	14 (35%)	6 (43%)	3 (38%)	2 (33%)	0 (0%)	0 (0%)	2 (100%)	1 (100%)
Fracture of occipital bone	13 (33%)	3 (21%)	5 (63%)	2 (33%)	0 (0%)	0 (0%)	2 (100%)	1 (100%)
Fracture of temporal	12 (30%)	4 (29%)	3 (38%)	2 (33%)	0 (0%)	0 (0%)	2 (100%)	1 (100%)
Fracture of parietal bone	10 (25%)	4 (29%)	4 (50%)	1 (17%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Fracture of tibia	10 (25%)	2 (14%)	4 (50%)	2 (33%)	1 (20%)	1 (25%)	0 (0%)	0 (0%)
Fracture of jawbone	9 (23%)	2 (14%)	3 (38%)	1 (17%)	0 (0%)	1 (25%)	1 (50%)	1 (100%)
Fracture of scapula	9 (23%)	3 (21%)	3 (38%)	0 (0%)	1 (20%)	0 (0%)	1 (50%)	1 (100%)
Fracture of thoracic vertebrae	8 (20%)	2 (14%)	1 (13%)	1 (17%)	3 (60%)	0 (0%)	1 (50%)	0 (0%)
Fracture of maxilla	8 (20%)	3 (21%)	2 (25%)	2 (33%)	0 (20%)	0 (0%)	1 (0%)	0 (0%)
Fracture of fibula	8 (20%)	3 (21%)	2 (25%)	2 (33%)	1 (0%)	0 (0%)	0 (50%)	0 (0%)
Fracture of nasal bone	7 (18%)	2 (14%)	1 (13%)	1 (17%)	0 (0%)	1 (25%)	2 (100%)	0 (0%)
Fracture of radius	7 (18%)	3 (21%)	4 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Fracture of ulna	6 (15%)	1 (14%)	3 (25%)	1 (0%)	0 (20%)	0 (0%)	1 (50%)	0 (0%)
Fracture of femur	6 (15%)	4 (29%)	1 (13%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)
Diaphragm rupture	6 (15%)	2 (7%)	2 (38%)	0 (17%)	1 (0%)	0 (0%)	1 (50%)	0 (0%)
Luxation of lumbar vertebrae	5 (13%)	3 (7%)	0 (25%)	1 (0%)	0 (40%)	0 (0%)	1 (0%)	0 (0%)
Luxation of coxofemoral joint	5 (13%)	1 (21%)	2 (0%)	0 (17%)	2 (0%)	0 (0%)	0 (50%)	0 (0%)
Fracture of cervical vertebrae	4 (10%)	0 (0%)	1 (13%)	1 (17%)	1 (20%)	0 (0%)	0 (0%)	1 (100%)
Fracture of humerus	4 (10%)	2 (14%)	1 (13%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)
Luxation of humeroradial joint	3 (8%)	0 (0%)	1 (13%)	1 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Fracture of clavicle	3 (8%)	0 (21%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0%)	0 (0%)
Fracture of lumbar vertebrae	3 (8%)	3 (0%)	0 (13%)	0 (17%)	0 (0%)	0 (0%)	0 (50%)	0 (0%)
Luxation of cervical vertebrae	2 (5%)	0 (0%)	0 (0%)	1 (0%)	0 (40%)	1 (0%)	0 (0%)	0 (0%)
Heart eventration	2 (5%)	1 (0%)	0 (13%)	0 (0%)	0 (0%)	0 (25%)	1 (0%)	0 (0%)
Fracture of coccygeal vertebrae	2 (5%)	0 (14%)	1 (0%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)
Fracture of zygomatic	2 (5%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Luxation of tibiotarsal joint	2 (5%)	1 (7%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (50%)	0 (0%)
Consolidated fracture of thoracic vertebrae	2 (5%)	0 (0%)	0 (0%)	0 (17%)	2 (0%)	0 (25%)	0 (0%)	0 (0%)
Fracture of sacrum	2 (5%)	2 (7%)	0 (0%)	0 (0%)	0 (20%)	0 (0%)	0 (0%)	0 (0%)
Fracture of calcaneum	1 (3%)	0 (0%)	0 (13%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Rupture of abdominal organs	1 (3%)	0 (0%)	0 (0%)	1 (0%)	0 (20%)	0 (0%)	0 (0%)	0 (0%)

Cont.

Laceration of cervical musculature	1 (3%)	0 (0%)	0 (13%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)
Luxation of ribs	1 (3%)	0 (0%)	0 (0%)	0 (17%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)
Comminute fracture of breastbone	1 (3%)	0 (0%)	1 (0%)	0 (0%)	0 (20%)	0 (0%)	0 (0%)	0 (0%)
Consolidated fracture of radius	1 (3%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (100%)
Luxation of thoracic vertebrae	1 (3%)	0 (7%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Fracture of lacrimal	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (50%)	1 (0%)
Consolidated fracture of coccygeal vertebrae	1 (3%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)
Fracture of epipubic bone	1 (3%)	0 (7%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)
Laceration of femoral musculature	1 (3%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)	0 (25%)	0 (0%)	0 (0%)
Luxation of sacroiliac joint	1 (3%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (50%)	0 (0%)
Luxation of coccygeal vertebrae	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (20%)	1 (0%)	0 (0%)	0 (0%)
Fracture of metatarsal bones	1 (3%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Luxation of atlantooccipital joint	1 (3%)	1 (0%)	0 (13%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Luxation of temporomandibular joint	1 (3%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pneumomediastinum	1 (3%)	1 (0%)	0 (0%)	0 (17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

CA=Carnívora; PI=Pilosa; RO=Rodentia; DI=Didelphimorphia; CI=Cingulata

3.2.4 **DISCUSSION**

The biomechanics of traumatic injuries (BTI) by MVC in pedestrians include primary injuries (caused by the first impact with the vehicle), secondary injuries (second impact with the vehicle), and tertiary (subsequent contact with the ground) (Saukko & Knight, 2016; Brooks, 2018). The height of the bumper (HB) is directly associated with fractures in the tibia, femur, and knee dislocations, and this is directly related to the distance from the ground to the bumper (Strandroth et al, 2011; Wang et al, 2019). According to international regulations, the HB in cars should range between 30-50 cm and 60 cm in trucks (https://www.grsproadsafety.org/). The human is plantigrade and has a bipedal gait, and the average height is between 160-170 cm. The bumper impacts directly on the tibia or knee in adults and on the thighs or pelvis in children; trauma is typically more severe in the latter (Di Maggio et al, 2006). In humans, MVC-TI patterns and most affected regions are (by decreasing order) lower extremities, abdomen, thorax, head-neck, and upper extremities (Peng & Bongard, 1999; AlEassa & Abu-Zidan, 2013). Comparatively, MVC-TI and patterns are poorly studied, including assessments in domestic dogs (Kolata & Johnston, 1975), wild mammals (Garcês, 2021), and Brazilian tapirs (Navas-Suárez et al, 2019); in the present study, the most affected segments were AP, TX, and HN, followed by EX. Most terrestrial mammals are quadrupeds with digitigrade, unguligrade, and plantigrade characteristics. The most used height measurement in quadruped animals is the height at withers (HW). In this study, the HW in large-size mammals varied between 61-120 cm, medium-size between 20-60 cm, and small-size less than 20 cm. Therefore, considering the car HB, it is expected that animals between 20-60 cm will present extensive lesions throughout the body and that small-sized mammals presented greater incidence of compressive lesions because of impact with the wheels (Klainbart et al, 2018). These assumptions were readily apparent in this study, with lesions over 80% frequency in AP, TX, and HN regions. By contrast, a lower incidence of injury in EX could be explained because extremities were typically lower than HB, which agrees with Garcês (Garcês, 2021).

Some authors speculate about pre-existing disease in wildlife may be a relevant predisposing cause that contributes to unnatural mortality events (Wobeser, 2013; Sánchez et al, 2018). Interestingly, in this study, just a reduced number of the animals

evaluated had pre-existing lesions that could have explained death or could potentially pose significant health concerns; and the vast majority of the animals had apparent good nutritional status. Therefore, our results do not lend support to this hypothesis. These results provide strong evidence that most of these animals were seemingly healthy otherwise.

As mentioned above, speed and type of vehicle are critical factors in understanding the extent and severity of injuries. Even though in humans it is recognized that high-speed collisions are usually fatal, collisions at speeds close to 10 km/h can also result in fatalities (Mackay, 1994; Eid & Abu-Zidan, 2007). Unfortunately, obtaining data on collision speed and vehicle type is not feasible in most circumstances. However, the speed limit in the highways represented in this study ranges between 80 to 110 km/h. In addition, very few road sections had speed control radars. Current road safety guidelines recommend that abrupt manoeuvring or extreme deceleration should be avoided if the driver encounters a small animal on the track. For these reasons, we believe that collision speeds, at least in small animals, very likely occurred at highways' speed limits and could explain the severity of lesions and frequency of fatal MVC-TI.

Blunt force (BF) injury is caused by an impact without acute penetration of the body cavities (Sharma & Holowaychuk, 2015). Thoracic BF was the most plausible cause of acute pulmonary hemodynamic disturbances and often coexisted with lung parenchyma rupture and rib fractures, as observed in previous accounts (Fighera et al, 2008). Haemothorax is also common in MVC in domestic dogs (Simpson et al, 2009; Intarapanich et al, 2016) and wild mammals (Garcês, 2021). We observed hemoperitoneum in 39% of the animals and it was consistently associated with visceral and/or vascular rupture. Moreover, hemoperitoneum is a severe and common complication of intra-abdominal injuries in dogs (Lisciandro et al, 2009; Simpson et al, 2009; Hoffberg et al, 2016).

Rupture of parenchymal organs and diaphragmatic rupture and herniation are very common findings in dogs and cats subject to MVC (Mackay, 1994; Fighera et al, 2008). In this study, visceral rupture was the second most frequent finding, typically involving the liver, kidney, spleen, and lung. Diaphragmatic rupture was also common.

These results are aligned yet present mild variations with previous studies in dogs (Fighera et al, 2008; Garcês, 2021). As argued for bone fractures, visceral rupture distribution likely relates to species anatomic characteristics, size, and locomotion type.

Myocardial rupture was observed at low prevalence in our cases, and it occurred consistently at the interventricular septum. Rupture of the interventricular septum has been described in human MVC (East, 1945; Moront et al, 1991; Harel et al, 1995). By contrast, despite accounts of myocardial injury in MVC dogs, rupture in the interventricular septum has not been noted (Kolata & Johnston, 1975.). In these cases, myocardial haemorrhage is common. The pathogeneses proposed would involve a) BF-Trauma, compression of the chest and crushing of the heart between the sternum and the spine with subsequent myocardial rupture, b) adjacent bony fracture and direct cardiac perforation, or c) a combination thereof (Symbas, 1976; Romanucci et al, 2019). We also detected aortic base rupture (without aneurysm or dissecting phenomenon) in some deer and capybaras.

Postpandrial (physiologic) gastric dilation was common in our dataset. Some authors report this finding in domestic dogs and speculate upon an increased vulnerability due to food-related drowsiness (Klainbart et al, 2018), analogous to postprandial fatigue in humans (Wells et al, 1997). Calorie-rich food intake is associated with a decreased alertness ought to redistribution of blood perfusion, from the cerebral to the mesenteric blood vessels, resulting in a cerebral hypoperfusion syndrome (Landström et al, 2001; Eicke et al, 2003; Bazar et al, 2004). Although this theory is not fully proven and we cannot draw any definitive conclusion on this matter, our data confirm a set of fatal MVC cases present evidence of recent foraging or postprandial status.

Nail fracture was a gross finding observed main in sloths/anteaters (n=30; 28%), and carnivores (n=23; 17%). The literature considered it a frequent injury of the extremities in carnivores, mainly in MVC (Munro & Munro, 2008; Ressel et al, 2016). However, there are no previous reports of this BFT-injury in specimens of the Pilosa order. In carnivores, it is considered that nail fractures are associated with the fact that the animal tries to hold on with its nails in a traumatic event, and due to the force of the

impact, nails end up fracturing. This explanation could also hold true for anteaters and sloth subject to MVC.

In MVC-collared anteaters (T. mexicana) in Costa Rica, the most frequent traumatic lesions occurred in cranial portions of the body (Arguedas et al, 2019). In this study, we examined two species of anteaters (M. tridactyla, T. tetradactyla) and MVC-TI occurred in AP, TX, HN, and EX. We surmise the height of collared anteaters is in the range of HB, which could have influenced the distribution and severity of the injuries observed. Additionally, head injuries were slightly higher in T. tetradactyla, which could relate to an agonistic behaviour in this species characterized by leaning on the hind limbs and being alert with the forelimbs to attack with the nails (Haddad Jr et al. 2014), or because of their low agility and relatively slow locomotion (Ribeiro et al, 2017). Therefore, if this species considers the vehicle a threat, anterior regions (TX, HN) would be prone to MVC-TI.

In opossums, MVC-skeletal injuries (rib and scapular fractures) tent to occur at the cranial portion of the skeleton (Gardner, 1982; Mead & Patterson, 2009). An explanation to how a tire blow would break one or two ribs without seriously damaging other hard and soft tissues was not apparent (Mead & Patterson, 2009). In this study, the possum species are smaller than the North American species, and we believe that the size might have influenced the distribution and severity of the lesions observed in these animals.

In armadillos, it was evident that thoracic and abdominal trauma was much more frequent than in the head and extremities. We believe that their anatomic characteristics may explain this since armadillos have resilient integumentary adaption (e.g., "bony carapace") that provides protection (Chen et al, 2011). Additionally, their anatomic features render them a robust body; some species can protect their head, neck, and limbs with the shell. Due to these characteristics and its reduced size, we believe that trauma is caused by compression with the vehicle's wheels since its height does not exceed 30 cm, thus avoiding the bumper. There are no reports of traumatic lesions due to MVC in this taxon; further research is warranted to better understand BTI in armadillos.

The general male:female ratio was 1.5, with significant differences in marsupials (6) and rodents (2.1); comparatively, deer had a 0.4 ratio. In some taxa, such as carnivores, males tend to have larger home range and foraging behaviours, which increases their dispersal capacity compared to females (Kolata et al, 1974). A study of molecular sexing in one of the regions surveyed here showed that mainly for anteaters (M. tridactyla and T. tetradactyla) and armadillos (E. sexcinctus), the proportion of males is higher (Barragan-Ruiz et al, 2021); in the same region, there are studies demonstrating that in giant anteaters living near roads (<2 km) there are higher crossing rates for males than females (Noonan et al, 2021). This greater dispersal, and in the case of anteater, the higher density of males in the studied regions may explain why males had an apparent greater risk to contact with the roads and, therefore, higher probability to MVC.

Post-mortem imaging studies, namely radiology, computerized tomography, or magnetic resonance in humans are widely used in MVC patients, allowing for detection of lesions otherwise not easily detectable upon gross examination. These studies also enable reconstruction of the collision using computational tools (Chatzaraki et al, 2018). We performed X-rays in a small portion of the cases. Typical findings in these cases were: small fractures involving the spinous processes of the vertebrae, vertebral and appendicular luxations, pneumothorax, pneumomediastinum, and projectiles. Some of these lesions could have gone unnoticed at necropsy. Use of advanced diagnostic imaging techniques in suspect MVC is highly indicated.

There is lack of information on the behavioural aspects of wildlife-MVC. A theoretical study suggested that for a wild animal to avoid a collision, successful detection of the vehicle, a threat assessment, and evasive behaviour are required; however, whether wildlife species recognize vehicles as threats remains uncertain (Lima et al, 2015). Studies in giant anteaters showed that individuals living near roads do not actively seek passage structures to crossroads, suggesting that this species needs crossing structures accompanied by fences leading to existing passages (Noonan et al, 2021). Our data do not allow us to draw any conclusion on this specific issue but they do confirm fatal MVC is a leading cause of death in Neotropical wildlife.

In summary, these results characterized and delineated the anatomopathologic features of MVC-TI in a large set of neotropical wild mammals from Brazil, providing scientific basis for pathologic understanding of fatal MVC-TI in wildlife in other geographic locations. Overall, we observed higher incidence of traumatic injuries in AP, TX and HN, in comparison to EX. Gross pathologic findings were common, including single or multiple bone fractures, visceral rupture, cutaneous/subcutaneous hemorrhage/hematoma, haemothorax, pulmonary haemorrhage, and hemoperitoneum; these findings represent the basis of a diagnosis of fatal MVC. Moreover, no distinct patterns of traumatic injury were readily evident across the different taxonomic groups. These results may aid clinicians performing emergency care in MVC patients and may be of value in pathologic and forensic investigations wherein MVC is deemed a likely contributor factor of death.

Conflict of Interest Statement

The authors declared no financial interest or other potential conflicts of interest in relation to the research, authorship or publication of this article. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplemental figure 1. A) *Puma concolor*. Cutaneous laceration in the right gluteal and scapular regions. B) *P. concolor*. Cranioencephalic polytraumatism with palate fracture. C) *Leopardus pardalis*. Craniofacial polytraumatism with left eye exophthalmia. D) *L. guttulus*. Nail fracture. E) *Galictis cuja*. Subcutaneous hemorrhage along the nuchal region. F) *Cerdocyon thous*. Hemorrhage in ventral subcutis of abdominal and thoracic regions. G) *C. thous*. Rupture of diaphragm with herniation of liver into the thoracic cavity. H) *P. jagouaroundii*. Eventration of heart and pulmonary lobes on the subcutaneous axillar region and hemorrhage. I) *C. thous*. Left hemi-hemothorax. J) *L. pardalis*. Hemoperitoneum. K) *C. thous*. Myocardial rupture along the interventricular septum. L) *P. concolor*. Rupture of hepatic parenchyma.
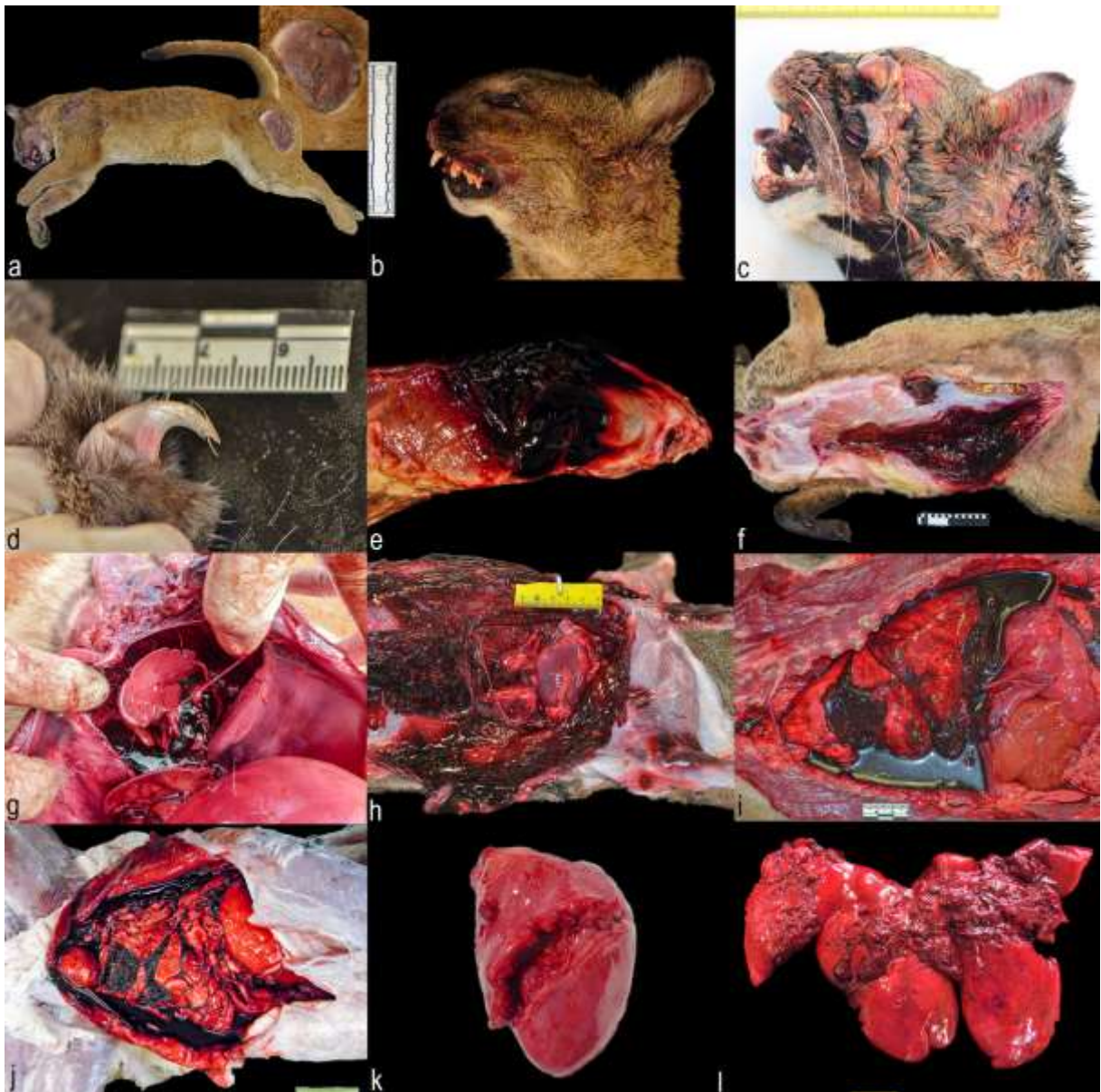
Supplemental figure 2. A) *Myrmecophaga tridactyla*. Note blood over the right eye. B) *M. tridactyla*. Foamy fluid oozing from the mouth and nostrils. C) *Dasypus novemcinctus*. Cervical hematoma. D) *Bradipus variegatus*. Craniofacial polytraumatism with exophthalmia of left eye. E) *D. novemcinctus*. Linear cutaneous laceration. F) *Tamandua tetradactyla*. Multifocal skull fracture with brain rupture and hemorrhage. G) *M. tridactyla*. Skin abrasions. H) *T. tetradactyla*. Rupture of claw. I) *B. variegatus*. Rupture of claw. J) *T. tetradactyla*. Subcutaneous emphysema in the right hindlimb. K) *T. tetradactyla*. Myocardial rupture. L) *Myrmecophaga tridactyla*. Hemoperitoneum.

Supplemental figure 3. A), B) and C). *Mazama gouazoubira*. Multifocal skin abrasions and lacerations. D) *M. gouazoubira*. Serosanguineous foamy fluid oozing from nares. E) *M. gouazoubira*. Metatarsal fracture. F) *M. gouazoubira*. Fractures of temporal and parietal bones with brain rupture and hemorrhage. G) and H). *M. gouazoubira*. Left hemi- hemothorax. I) *Tapirus terrestris*. Stools and ingesta over thoracic and peritoneal serosae. J) *M. gouazoubira*. Rupture of hepatic parenchyma. K) *M. gouazoubira*. Pulmonary perforation secondary to rib fracture. L) *M. gouazoubira*. Cranioencephalic trauma with skull and brain fractures.
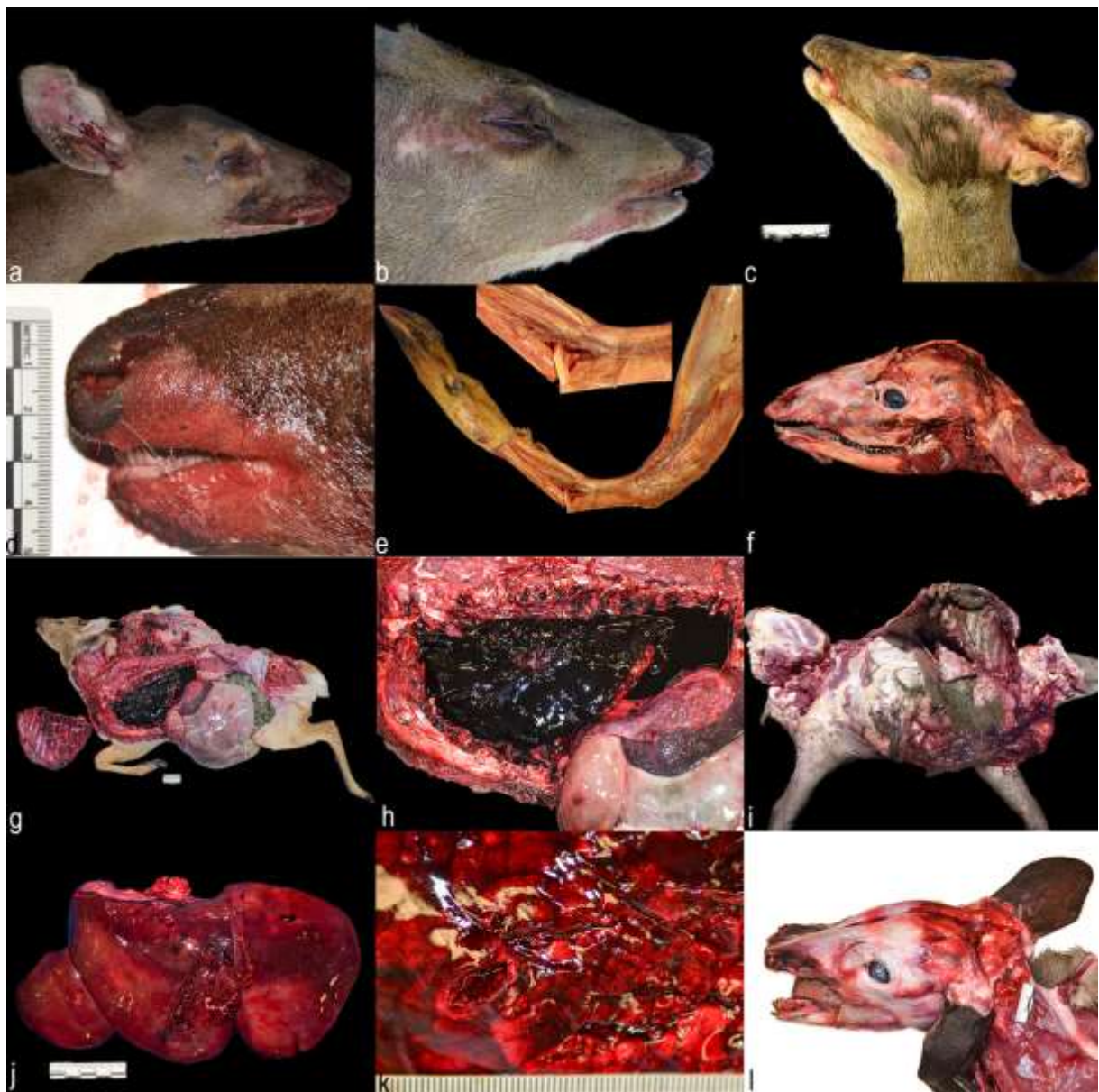
Supplementary figure 4. A) *Hydrochoerus hydrochaeris*. Skin abrasions and contusions. B) *Cuniculus paca*. Facial hemorrhage (oral cavity, nostrils). C) *Dasyprocta azarae*. Craniofacial polytraumatism with exophthalmia of right eye. D) *H. hydrochaeris*. Skin abrasion with hemorrhage in right foreleg. E) *Didelphis aurita*. Partial evisceration. F) *D. aurita*. *Didelphis aurita*. Nasal passage congestion and hemorrhage. E) *C. paca*. Zygomatic bone fracture. F) *H. hydrochaeris*. Marked subcutaneous hemorrhage in femoral region. G) *C. paca.* Abundant serosanguineous foamy fluid within trachea. H) *D. aurita*. Rip fracture with rupture of intercostal muscle. H) *H. hydrochaeris*. Aortic intimal rupture. I) *Coendou spinosus*. Hemopericardium (cardiac tamponade).
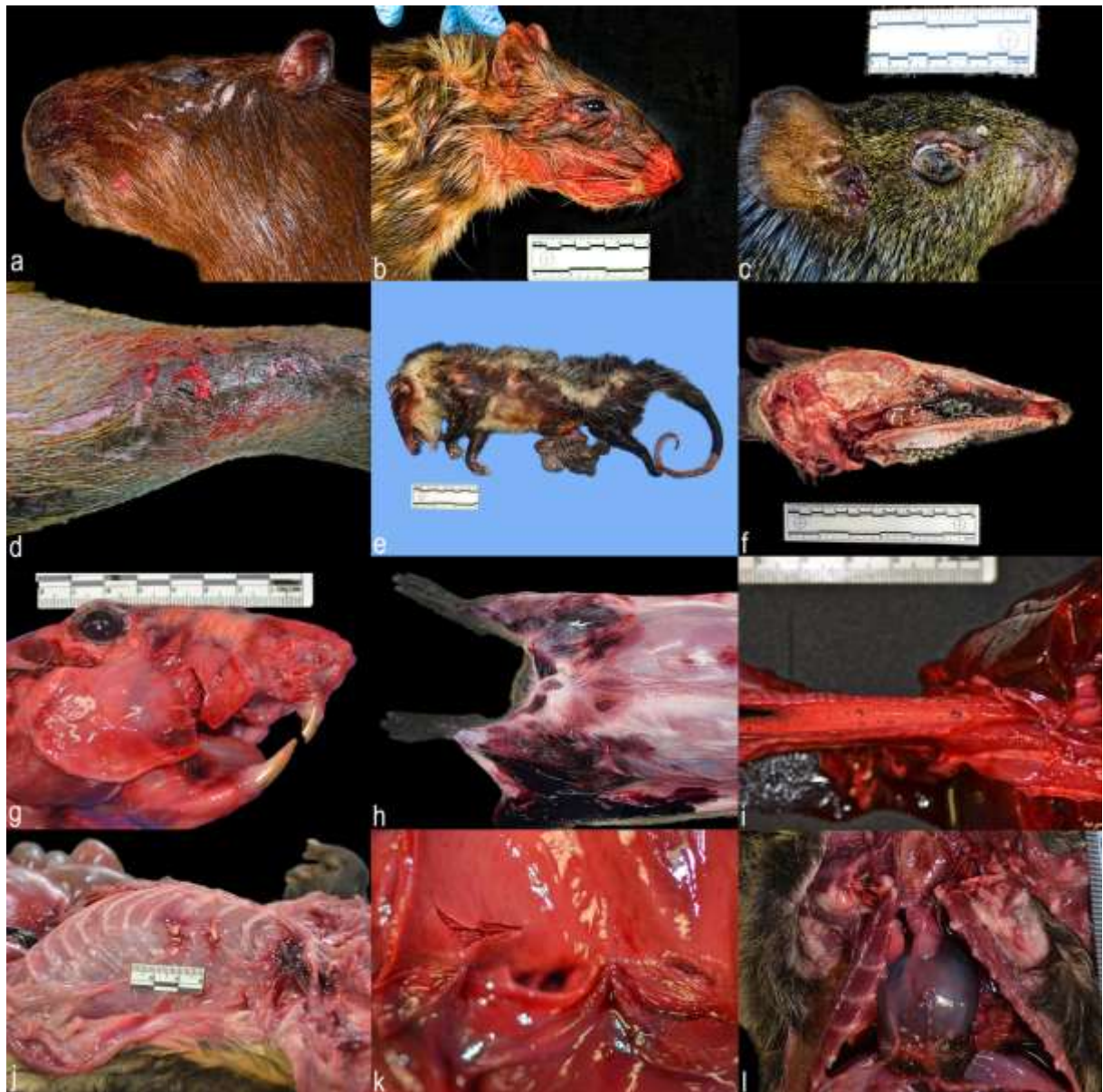
Supplemental figure 5. A) *Alouatta guariba clamitans*. Fracture of canines and jaw. B) *A. caraya.* Craniofacial polytraumatism with exophthalmia of right eye. C) *A. caraya.* Note in the left image pectoral region with vehicle paint debris and wheel marks, right image normal. D) andE) *Callithrix aurita*. Subcutaneous hematoma in inguinal region, and multiple fracture of pelvis with hemorrhage. F) *A. g, clamitans.* Subcutaneous hemorrhage along the cranial region. G) *A. caraya.* Ingesta in subcutis of the extremities. H) *A. caraya.* Complete fracture of radius. I) *A. caraya.* Complete luxation of elbow. J) *A. g. clamitans.* Bilateral hemothorax. K) *C. aurita.* Pulmonary congestion and hemorrhage. L) *A. caraya.* Rupture of hepatic parenchyma.
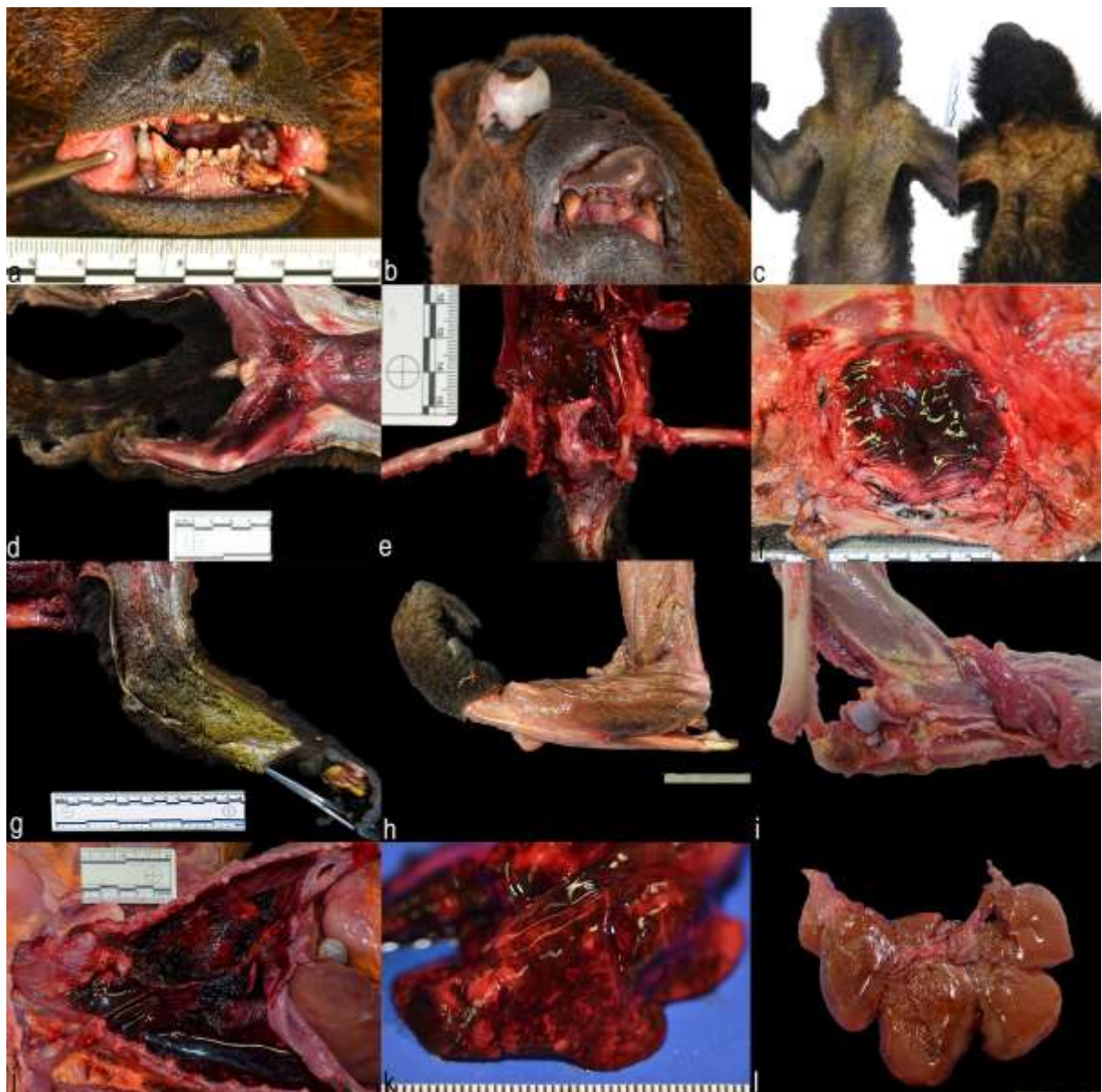
Supplemental table 1. Biological characteristics of the species of wild mammals

Class	Locomotion	Activity	Diet	Social behavior	IUCN	BRASIL	CITES	Population trend	Habitat loss classification	Total
					Conservation trends					
Artiodactyla (n=21)										
Brown Brocket Deer (*Mazama gouazoubira*)	Terrestrial	Diurnal	Herbivore	Solitary	LC	LC	-	Decreasing	Resilient	21
Carnivora (n=138)										
Bush Dog (*Speothos venaticus*)	Terrestrial	Diurnal	Carnivore	Gregary	NT	VU	I	Decreasing	Sensitive	1
Crab-eating Fox (*Cerdocyon thous*)	Terrestrial	Nocturnal	Omnivore	Solitary	LC	LC	II	Stable	Resilient	44
Crab-eating Raccoon (*Procyon cancrivorus*)	Scansorial	Nocturnal	Omnivore	Solitary	LC	LC	-	Decreasing	Resilient	20
Hoary Fox (*Lycalopex vetulus*)	Terrestrial	Nocturnal	Omnivore	Solitary	LC	VU	-	Decreasing	Sensitive	3
Jaguarundi (*Herpailurus yagouaroundi*)	Terrestrial	Both	Carnivore	Solitary	LC	VU	II	Decreasing	Resilient	12
Lesser Grison (*Galictis cuja*)	Terrestrial	Both	Carnivore	Small groups	LC	LC	-	Unknown	Resilient	8
Maned Wolf (*Chrysocyon brachyurus*)	Terrestrial	Nocturnal	Omnivore	Solitary	NT	VU	II	Unknown	Sensitive	5
Margay (*Leopardus wiedii*)	Scansorial	Nocturnal	Carnivore	Solitary	NT	VU	II	Decreasing	Sensitive	2
Neotropical Otter (*Lontra longicaudis*)	Semiaquatic	Both	Carnivore	Solitary	NT	NT	I	Decreasing	Resilient	5
Ocelot (*Leopardus pardalis*)	Scansorial	Nocturnal	Carnivore	Solitary	LC	LC	I	Decreasing	Sensitive	13
Puma (*Puma concolor*)	Terrestrial	Both	Carnivore	Solitary	LC	VU	I	Decreasing	Resilient	4
South American Coati (*Nasua nasua*)	Terrestrial	Diurnal	Omnivore	Gregary	LC	LC	III	Decreasing	Resilient	12
Southern Tiger Cat (*Leopardus guttulus*)	Scansorial	Nocturnal	Carnivore	Solitary	VU	VU	I	Decreasing	Resilient	7
Striped Hog-nosed Skunk (*Conepatus semistriatus*)	Terrestrial	Nocturnal	Carnivore	Solitary	LC	LC	-	Unknown	Resilient	1
Tayra (*Eira barbara*)	Scansorial	Nocturnal	Omnivore	Solitary	LC	LC	-	Decreasing	Resilient	1
Cingulata (n=50)										
Giant Armadillo (*Priodontes maximus*)	Semiphosorial	Nocturnal	Insectivore	Solitary	VU	VU	I	Decreasing	Sensitive	2
Greater Naked-tailed Armadillo (*Cabassous tatouay*)	Semiphosorial	Nocturnal	Insectivore	Solitary	LC	DD	-	Unknown	Resilient	1
Nine-banded Armadillo (*Dasypus novemcinctus*)	Semiphosorial	Nocturnal	Insectivore	Solitary	LC	LC	-	Stable	Resilient	24

Cont.

Southern Naked-Tailed Armadillo (*Cabassous unicinctus*)	Semiphosorial	Nocturnal	Insectivore	Solitary	LC	LC	-	Unknown	Resilient	3
Yellow Armadillo (*Euphractus sexcinctus*)	Semiphosorial	Diurnal	Omnivore	Solitary	LC	LC	-	Stable	Resilient	20

Didelphimorphia (n=21)

Brazilian Common Opossum (*Didelphis aurita*)	Scansorial	Nocturnal	Omnivore	Solitary	LC	LC	-	Stable	Resilient	14
Gray and black four-eyed opossum (*Philander frenatus*)	Scansorial	Nocturnal	Omnivore	Solitary	LC	LC	-	Unknown	Sensitive	2
White-eared Opossum (*Didelphis albiventris*)	Scansorial	Nocturnal	Omnivore	Solitary	LC	LC	-	Stable	Resilient	5

Lagomorpha (n=3)

Brazilian cottontail (*Sylvilagus brasiliensis*)	Terrestrial	Both	Herbivore	Solitary	EN	LC	-	Decreasing	Resilient	2
European hare (*Lepus europaeus*)	Terrestrial	Nocturnal	Herbivore	Solitary	-	-	-	Exotic	Resilient	1

Perissodactyla (n=36)

Lowland Tapir (*Tapirus terrestris*)	Terrestrial	Both	Herbivore	Solitary	VU	VU	II	Decreasing	Sensitive	36

Pilosa (n=106)

Brown-throated Sloth (*Bradypus variegatus*)	Arboreal	Diurnal	Herbivore	Solitary	LC	LC	II	Unknown	Sensitive	5
Giant Anteater (*Myrmecophaga tridactyla*)	Terrestrial	Both	Insectivore	Solitary	VU	VU	II	Decreasing	Sensitive	61
Southern Tamandua (*Tamandua tetradactyla*)	Scansorial	Nocturnal	Insectivore	Solitary	LC	LC	-	Unknown	Resilient	40

Primates (n=20)

Bearded Capuchin (*Sapajus libidinosus*)	Arboreal	Diurnal	Omnivore	Gregary	NT	NT	-	Decreasing	Sensitive	2
Black-and-gold Howler Monkey (*Alouatta caraya*)	Arboreal	Diurnal	Herbivore	Gregary	NT	NT	-	Decreasing	Sensitive	5
Black-horned Capuchin (*Sapajus nigritus*)	Arboreal	Diurnal	Omnivore	Gregary	NT	NT	-	Decreasing	Resilient	2
Buffy-tufted-ear Marmoset (*Callithrix aurita*)	Arboreal	Diurnal	Omnivore	Gregary	EN	EN	I	Decreasing	Sensitive	4
Southern Brown Howler Monkey (*Alouatta guariba ssp. Clamitans*)	Arboreal	Diurnal	Herbivore	Gregary	VU	VU	-	Decreasing	Resilient	7

Cont.

Rodentia (n=35)

Agouti (*Cuniculus paca*)	Terrestrial	Both	Herbivore	Solitary	LC	LC	-	Stable	Resilient	1
Azara's Agouti (*Dasyprocta azarae*)	Terrestrial	Both	Herbivore	Solitary	DD	LC	-	Decreasing	Sensitive	2
Bamboo Rat (*Kannabateomys amblyonyx*)	Arboreal	Nocturnal	Omnivore	Small groups	LC	LC	-	Unknown	Resilient	1
Brazilian Guinea Pig (*Cavia aperea*)	Terrestrial	Both	Herbivore	Small groups	LC	LC	-	Stable	Resilient	6
Brazilian squirrel (*Guerlinguetus brasiliensis*)	Scansorial	Diurnal	Herbivore	Small groups	-	LC	-	Unknown	Resilient	1
Brown rat (*Rattus norvegicus*)	Terrestrial	Nocturnal	Omnivore	Gregary	-	-	-	Exotic	Resilient	2
Capybara (*Hydrochoerus hydrochaeris*)	Semiaquatic	Both	Herbivore	Gregary	LC	LC	-	Stable	Resilient	12
Coypu (*Myocastor coypus*)	Semiaquatic	Nocturnal	Herbivore	Gregary	LC	LC	-	Decreasing	Resilient	2
Hairy dwarf porcupine (*Coendou spinosus*)	Arboreal	Nocturnal	Omnivore	Solitary	LC	LC	-	Unknown	Resilient	8
									Total	**430**

Supplemental table 2. Organ/tissues evaluated and frequency of each category.

Tissue	Categories	Carnivora	Pilosa	Cingulata	Perissodactyla	Rodentia	Artiodactyla	Didelphimorphia	Primates	Total
	Total	125	99	47	34	28	21	18	19	394
	MVCTI	115 (92%)	99 (100%)	46 (98%)	27 (79%)	25 (89%)	20 (95%)	14 (78%)	17 (89%)	366 (93%)
Lungs	Non-MVCTI	78 (62%)	27 (27%)	37 (79%)	27 (79%)	17 (61%)	4 (19%)	12 (67%)	10 (53%)	213 (54%)
	NHF	2 (2%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)	3 (1%)
	Autolysis	24 (19%)	6 (6%)	6 (13%)	2 (6%)	2 (7%)	2 (10%)	7 (39%)	6 (32%)	56 (14%)
	Total	112	84	31	32	27	21	15	19	344
	MVCTI	6 (5%)	12 (14%)	1 (3%)	2 (6%)	1 (4%)	0 (0%)	1 (7%)	2 (11%)	25 (7%)
Heart	Non-MVCTI	17 (15%)	13 (15%)	3 (10%)	9 (28%)	5 (19%)	2 (10%)	2 (13%)	3 (16%)	54 (16%)
	NHF	70 (63%)	59 (70%)	24 (77%)	20 (63%)	19 (70%)	16 (76%)	7 (47%)	15 (79%)	232 (67%)
	Autolysis	25 (22%)	7 (8%)	4 (13%)	3 (9%)	3 (11%)	3 (14%)	5 (33%)	2 (11%)	53 (15%)
	Total	102	93	29	32	27	19	15	17	337
	MVCTI	8 (8%)	31 (33%)	4 (14%)	7 (22%)	1 (4%)	0 (0%)	0 (0%)	1 (6%)	52 (15%)
Liver	Non-MVCTI	33 (32%)	40 (43%)	13 (45%)	18 (56%)	12 (44%)	7 (37%)	6 (40%)	13 (76%)	142 (42%)
	NHF	23 (23%)	29 (31%)	9 (31%)	6 (19%)	3 (11%)	4 (21%)	1 (7%)	2 (12%)	78 (23%)
	Autolysis	61 (60%)	22 (24%)	9 (31%)	6 (19%)	14 (52%)	8 (42%)	9 (60%)	3 (18%)	135 (40%)
	Total	110	92	28	31	27	19	11	15	336
	MVCTI	13 (12%)	30 (33%)	5 (18%)	12 (39%)	3 (11%)	1 (5%)	0 (0%)	1 (7%)	65 (19%)
Kidney	Non-MVCTI	18 (16%)	15 (16%)	6 (21%)	8 (26%)	7 (26%)	1 (5%)	4 (36%)	6 (40%)	65 (19%)
	NHF	18 (16%)	30 (33%)	11 (39%)	11 (35%)	4 (15%)	1 (5%)	0 (0%)	3 (20%)	79 (24%)
	Autolysis	70 (64%)	30 (33%)	8 (29%)	7 (23%)	16 (59%)	17 (89%)	9 (82%)	7 (47%)	167 (50%)
	Total	100	78	22	30	20	19	9	11	291
	MVCTI	11 (11%)	22 (28%)	5 (23%)	18 (60%)	2 (10%)	3 (16%)	1 (11%)	0 (0%)	62 (21%)
Spleen	Non-MVCTI	30 (30%)	38 (49%)	15 (68%)	10 (33%)	11 (55%)	9 (47%)	0 (0%)	5 (45%)	118 (41%)
	NHF	16 (16%)	22 (28%)	0 (0%)	6 (20%)	1 (5%)	3 (16%)	1 (11%)	3 (27%)	52 (18%)
	Autolysis	54 (54%)	13 (17%)	5 (23%)	4 (13%)	7 (35%)	5 (26%)	7 (78%)	3 (27%)	100 (34%)
	Total	96	75	20	24	20	14	14	14	280
	MVCTI	4 (4%)	18 (24%)	2 (10%)	1 (4%)	0 (0%)	0 (0%)	1 (7%)	0 (0%)	26 (9%)
Trachea	Non-MVCTI	5 (5%)	3 (4%)	3 (15%)	11 (46%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	22 (8%)
	NHF	80 (83%)	54 (72%)	15 (75%)	11 (46%)	19 (95%)	14 (100%)	11 (79%)	14 (100%)	221 (79%)
	Autolysis	8 (8%)	1 (1%)	0 (0%)	1 (4%)	1 (5%)	0 (0%)	2 (14%)	0 (0%)	13 (5%)
	Total	82	85	18	30	21	6	4	10	258
	MVCTI	1 (1%)	2 (2%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (2%)
Small intestine	Non-MVCTI	30 (37%)	32 (38%)	9 (50%)	18 (60%)	3 (14%)	1 (17%)	2 (50%)	6 (60%)	101 (39%)
	NHF	24 (29%)	34 (40%)	6 (33%)	10 (33%)	11 (52%)	3 (50%)	1 (25%)	5 (50%)	94 (36%)
	Autolysis	45 (55%)	38 (45%)	4 (22%)	3 (10%)	10 (48%)	3 (50%)	3 (75%)	2 (20%)	110 (43%)
Esophagus	Total	79	68	16	12	19	13	12	12	234

	MVCTI	4 (5%)	17 (25%)	2 (13%)	1 (8%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)	25 (11%)
	Non-MVCTI	10 (13%)	5 (7%)	1 (6%)	2 (17%)	2 (11%)	0 (0%)	0 (0%)	1 (8%)	21 (9%)
	NHF	61 (77%)	46 (68%)	13 (81%)	8 (67%)	16 (84%)	13 (100%)	11 (92%)	11 (92%)	182 (78%)
	Autolysis	8 (10%)	2 (3%)	0 (0%)	1 (8%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	12 (5%)
	Total	61	62	26	21	13	16	10	10	222
	MVCTI	2 (3%)	4 (6%)	1 (4%)	2 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (4%)
Tongue	Non-MVCTI	18 (30%)	23 (37%)	13 (50%)	10 (48%)	2 (15%)	5 (31%)	2 (20%)	4 (40%)	78 (35%)
	NHF	35 (57%)	37 (60%)	13 (50%)	10 (48%)	10 (77%)	11 (69%)	6 (60%)	6 (60%)	130 (59%)
	Autolysis	6 (10%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)	3 (30%)	0 (0%)	10 (5%)
	Total	57	63	15	29	9	0	7	7	189
	MVCTI	1 (2%)	2 (3%)	0 (0%)	2 (7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (3%)
Stomach	Non-MVCTI	17 (30%)	22 (35%)	5 (33%)	8 (28%)	0 (0%)	0 (0%)	1 (14%)	1 (14%)	54 (29%)
	NHF	25 (44%)	34 (54%)	7 (47%)	17 (59%)	6 (67%)	0 (0%)	2 (29%)	6 (86%)	97 (51%)
	Autolysis	19 (33%)	10 (16%)	3 (20%)	2 (7%)	3 (33%)	0 (0%)	4 (57%)	0 (0%)	43 (23%)
	Total	51	46	16	11	14	16	8	9	172
	MVCTI	13 (25%)	18 (39%)	3 (19%)	3 (27%)	5 (36%)	4 (25%)	1 (13%)	5 (56%)	52 (30%)
Skeletal muscle	Non-MVCTI	5 (10%)	12 (26%)	6 (38%)	0 (0%)	0 (0%)	1 (6%)	2 (25%)	0 (0%)	26 (15%)
	NHF	25 (49%)	22 (48%)	7 (44%)	6 (55%)	7 (50%)	12 (75%)	5 (63%)	4 (44%)	89 (52%)
	Autolysis	9 (18%)	2 (4%)	0 (0%)	2 (18%)	3 (21%)	3 (19%)	1 (13%)	0 (0%)	20 (12%)
	Total	47	58	12	16	11	10	0	10	165
	MVCTI	8 (17%)	31 (53%)	5 (42%)	2 (13%)	1 (9%)	1 (10%)	0 (0%)	2 (20%)	50 (30%)
Adrenal glands	Non-MVCTI	12 (26%)	17 (29%)	2 (17%)	9 (56%)	2 (18%)	0 (0%)	0 (0%)	2 (20%)	44 (27%)
	NHF	18 (38%)	15 (26%)	6 (50%)	5 (31%)	4 (36%)	6 (60%)	0 (0%)	4 (40%)	59 (36%)
	Autolysis	15 (32%)	6 (10%)	1 (8%)	1 (6%)	5 (45%)	3 (30%)	0 (0%)	2 (20%)	34 (21%)
	Total	59	40	12	11	7	10	9	9	158
	MVCTI	2 (3%)	3 (8%)	1 (8%)	1 (9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7 (4%)
Urinary bladder	Non-MVCTI	2 (3%)	0 (0%)	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (2%)
	NHF	38 (64%)	33 (83%)	9 (75%)	10 (91%)	6 (86%)	8 (80%)	2 (22%)	7 (78%)	113 (72%)
	Autolysis	17 (29%)	4 (10%)	1 (8%)	0 (0%)	1 (14%)	2 (20%)	7 (78%)	4 (44%)	37 (23%)
	Total	53	39	8	20	5	5	4	6	141
	MVCTI	1 (2%)	5 (13%)	0 (0%)	8 (40%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	14 (10%)
Lymph node	Non-MVCTI	16 (30%)	29 (74%)	6 (75%)	10 (50%)	0 (0%)	0 (0%)	0 (0%)	3 (50%)	64 (45%)
	NHF	14 (26%)	8 (21%)	1 (13%)	7 (35%)	4 (80%)	2 (40%)	1 (25%)	3 (50%)	41 (29%)
	Autolysis	29 (55%)	3 (8%)	1 (13%)	1 (5%)	2 (40%)	4 (80%)	3 (75%)	1 (17%)	45 (32%)
	Total	32	47	6	26	9	10	0	8	138
	MVCTI	8 (25%)	19 (40%)	3 (50%)	8 (31%)	0 (0%)	1 (10%)	0 (0%)	2 (25%)	41 (30%)
Brain	Non-MVCTI	4 (13%)	6 (13%)	2 (33%)	1 (4%)	1 (11%)	2 (20%)	0 (0%)	1 (13%)	17 (12%)
	NHF	13 (41%)	23 (49%)	3 (50%)	16 (62%)	4 (44%)	3 (30%)	0 (0%)	3 (38%)	65 (47%)
	Autolysis	10 (31%)	4 (9%)	0 (0%)	2 (8%)	4 (44%)	5 (50%)	0 (0%)	3 (38%)	28 (20%)
Testicle	Total	45	23	11	6	14	5	9	8	121

	MVCTI	2 (4%)	0 (0%)	0 (0%)	1 (17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (2%)
	Non-MVCTI	40 (89%)	14 (61%)	9 (82%)	2 (33%)	11 (79%)	5 (100%)	5 (56%)	7 (88%)	93 (77%)
	NHF	3 (7%)	4 (17%)	3 (27%)	3 (50%)	3 (21%)	0 (0%)	3 (33%)	1 (13%)	20 (17%)
	Autolysis	2 (4%)	5 (22%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (22%)	0 (0%)	9 (7%)
	Total	32	59	9	8	5	1	0	2	117
Pancreas	MVCTI	0 (0%)	12 (20%)	4 (44%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	16 (14%)
	Non-MVCTI	1 (3%)	2 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	4 (3%)
	NHF	21 (66%)	37 (63%)	5 (56%)	6 (75%)	2 (40%)	1 (100%)	0 (0%)	0 (0%)	72 (62%)
	Autolysis	11 (34%)	12 (20%)	0 (0%)	2 (25%)	3 (60%)	0 (0%)	0 (0%)	1 (50%)	30 (26%)
	Total	35	26	7	10	9	6	1	3	97
Diaphragm	MVCTI	10 (29%)	5 (19%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	15 (15%)
	Non-MVCTI	5 (14%)	6 (23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	11 (11%)
	NHF	21 (60%)	16 (62%)	7 (100%)	9 (90%)	8 (89%)	6 (100%)	0 (0%)	3 (100%)	70 (72%)
	Autolysis	2 (6%)	1 (4%)	0 (0%)	1 (10%)	1 (11%)	0 (0%)	1 (100%)	0 (0%)	6 (6%)
	Total	31	16	2	12	10	5	3	6	87
Carotid	Non-MVCTI	0 (0%)	0 (0%)	0 (0%)	4 (33%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	5 (6%)
	NHF	30 (97%)	16 (100%)	2 (100%)	8 (67%)	9 (90%)	4 (80%)	3 (100%)	6 (100%)	80 (92%)
	Autolysis	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	2 (2%)
	Total	12	51	12	1	1	0	4	2	83
Salivary gland	MVCTI	0 (0%)	0 (0%)	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)
	Non-MVCTI	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)
	NHF	9 (0%)	47 (0%)	10 (0%)	1 (0%)	0 (0%)	0 (0%)	4 (0%)	2 (0%)	73 (2%)
	Autolysis	2 (17%)	4 (8%)	2 (17%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	9 (11%)
	Total	17	33	8	1	3	8	2	8	80
Skin	Non-MVCTI	3 (18%)	7 (21%)	4 (50%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	1 (13%)	16 (20%)
	NHF	12 (71%)	26 (79%)	4 (50%)	1 (100%)	3 (100%)	7 (88%)	1 (50%)	7 (88%)	61 (76%)
	Autolysis	3 (18%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	5 (6%)
	Total	39	21	7	0	4	0	1	3	76
Large intestine	Non-MVCTI	13 (33%)	2 (10%)	1 (14%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	17 (22%)
	NHF	11 (28%)	12 (57%)	6 (86%)	0 (0%)	1 (25%)	0 (0%)	1 (100%)	2 (67%)	33 (43%)
	Autolysis	19 (49%)	7 (33%)	1 (14%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	1 (33%)	31 (41%)
	Total	13	31	2	0	1	3	0	6	56
Cerebellum	MVCTI	2 (15%)	10 (32%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	12 (21%)
	Non-MVCTI	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
	NHF	9 (69%)	21 (68%)	2 (100%)	0 (0%)	1 (100%)	2 (67%)	0 (0%)	4 (67%)	39 (70%)
	Autolysis	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)	0 (0%)	2 (33%)	4 (7%)
	Total	22	6	1	0	8	2	4	5	50
Jugular	MVCTI	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
	NHF	21 (95%)	6 (100%)	1 (100%)	0 (0%)	7 (88%)	2 (100%)	4 (100%)	5 (100%)	48 (96%)
	Autolysis	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)

	Total	19	12	2	0	0	1	1	1	36
Lymph node, mesenteric	MVCTI	2 (11%)	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (8%)
	Non-MVCTI	8 (42%)	9 (75%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	19 (53%)
	NHF	1 (5%)	2 (17%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	5 (14%)
	Autolysis	10 (53%)	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	12 (33%)
	Total	2	0	0	25	0	0	0	0	27
Colon	Non-MVCTI	1 (50%)	0 (0%)	0 (0%)	9 (36%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	10 (37%)
	NHF	1 (50%)	0 (0%)	0 (0%)	16 (64%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	17 (63%)
Uterus	Total	6	6	1	6	2	3	1	2	27
	NHF	6 (100%)	6 (100%)	1 (100%)	6 (100%)	2 (100%)	3 (100%)	1 (100%)	2 (100%)	27 (100%)
	Total	9	4	5	0	2	1	0	1	22
Thymus	NHF	8 (89%)	4 (100%)	5 (100%)	0 (0%)	2 (100%)	1 (100%)	0 (0%)	1 (100%)	21 (95%)
	Autolysis	2 (22%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (9%)
	Total	7	3	2	0	1	1	1	3	19
Thyroid	NHF	5 (71%)	2 (67%)	2 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	3 (100%)	15 (79%)
	Autolysis	2 (29%)	1 (33%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	4 (21%)
	Total	4	0	1	0	1	3	2	2	13
Epiglottis	Non-MVCTI	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)
	NHF	4 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	3 (100%)	2 (100%)	2 (100%)	12 (92%)
	Total	0	0	0	0	0	13	0	0	13
Reticulum	NHF	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	12 (92%)	0 (0%)	0 (0%)	12 (92%)
	Autolysis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)	0 (0%)	1 (8%)
	Total	0	0	0	0	0	13	0	0	13
Rumen	NHF	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	11 (85%)	0 (0%)	0 (0%)	11 (85%)
	Autolysis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (15%)	0 (0%)	0 (0%)	2 (15%)
	Total	2	1	1	0	4	1	0	2	11
Penis	Non-MVCTI	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (9%)
	NHF	1 (50%)	1 (100%)	1 (100%)	0 (0%)	4 (100%)	1 (100%)	0 (0%)	2 (100%)	10 (91%)
	Total	0	0	0	0	0	10	0	0	10
Abomasum	NHF	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (90%)	0 (0%)	0 (0%)	9 (90%)
	Autolysis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	1 (10%)
Ovary	Total	6	2	0	0	1	0	0	1	10
	NHF	6 (100%)	2 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	10 (100%)
	Total	0	0	0	0	0	9	0	0	9
Omasum	NHF	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	8 (89%)	0 (0%)	0 (0%)	8 (89%)
	Autolysis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (11%)	0 (0%)	0 (0%)	1 (11%)
Spine	Total	3	2	0	0	0	3	0	0	8
	NHF	3 (100%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)	8 (100%)
Cecum	Total	1	0	0	5	0	0	0	0	6
	Non-MVCTI	0 (0%)	0 (0%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (33%)

Cont.

	NHF	0 (0%)	0 (0%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (33%)
	Autolysis	1 (100%)	0 (0%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (50%)
Eye	Total	3	0	0	0	2	1	0	0	6
	NHF	3 (100%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	1 (100%)	0 (0%)	0 (0%)	6 (100%)
	Total	0	1	0	0	0	3	0	1	5
Bone Marrow	NHF	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)	0 (0%)	1 (100%)	3 (60%)
	Autolysis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (67%)	0 (0%)	0 (0%)	2 (40%)
	Total	4	0	0	0	0	0	0	0	4
Adipose tissue	Non-MVCTI	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)
	NHF	3 (75%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (75%)
	Total	2	1	0	0	0	0	0	0	4
Duodenum	Non-MVCTI	1 (50%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (50%)
	Autolysis	1 (50%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (75%)
	Total	2	1	0	0	0	1	0	0	4
Tonsil	NHF	2 (100%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (75%)
	Autolysis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (25%)
Epididymis	Total	1	0	0	2	0	0	0	0	3
	Non-MVCTI	1 (100%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (100%)
Mammary gland	Total	0	0	0	2	0	1	0	0	3
	Non-MVCTI	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (67%)
	NHF	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (33%)
Parathyroid	Total	3	0	0	0	0	0	0	0	3
	NHF	2 (67%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (67%)
	Autolysis	1 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)
Prostate	Total	2	0	0	0	1	0	0	0	3
	NHF	1 (50%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	2 (67%)
	Autolysis	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)
Larynx	Total	2	0	0	0	0	0	0	0	2
	NHF	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)

4 CHAPTER III: HEALTH STUDIES IN ROADKILL SPECIMENS

4.1 PULMONARY ADIASPIROMYCOSIS IN ARMADILLOS KILLED BY MOTOR VEHICLE COLLISIONS IN BRAZIL
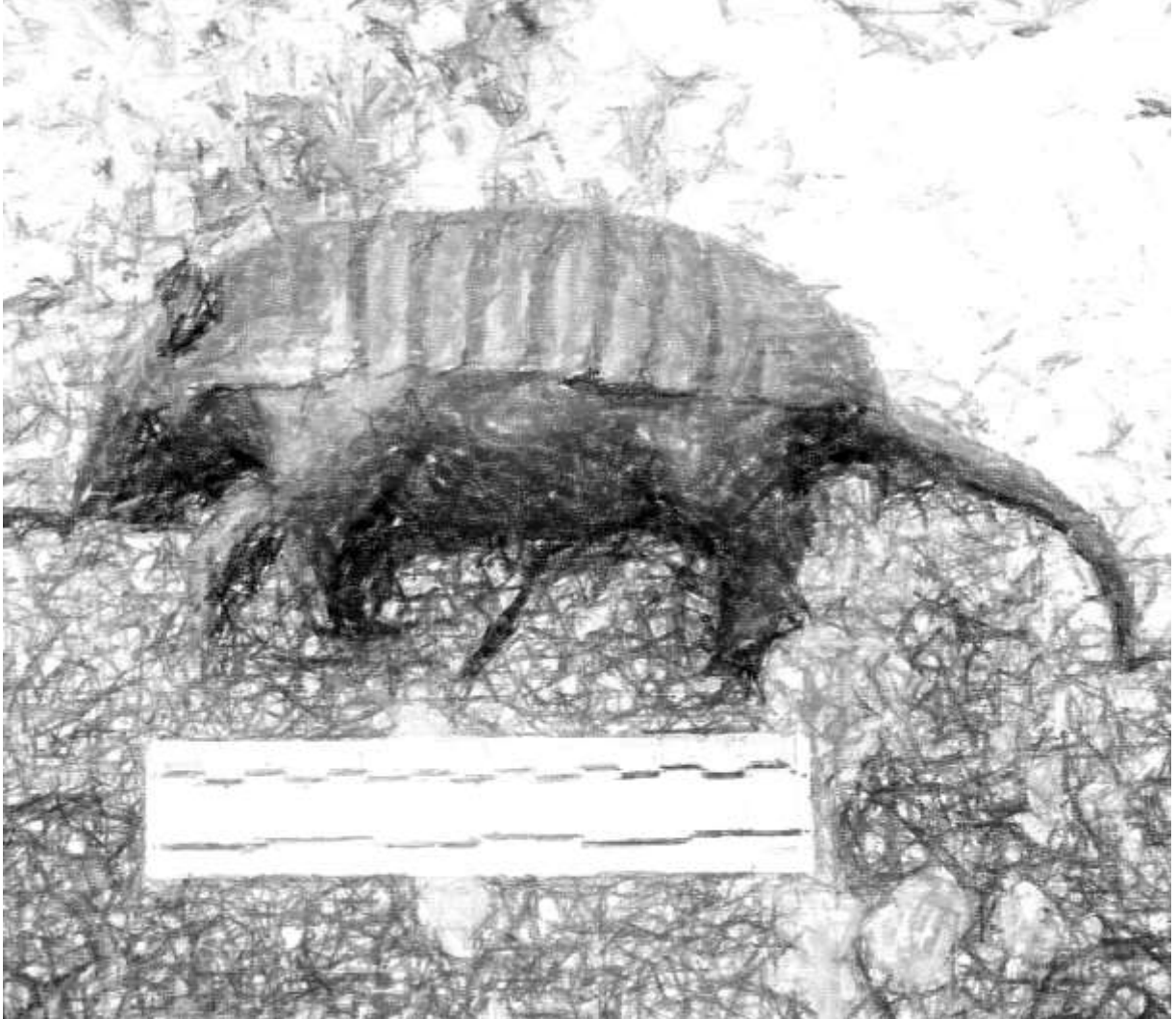
Authors: Navas-Suárez, P. E.; Sacristán, C.; Díaz-Delgado, J.; Yogui, D. R.; Alves, M. H.; Fuentes-Castillo, D.; Ospina-Pinto, M.; Zamana, R. R.; Desbiez A. L. J.; Catão-Dias, J. L.

4.1.1 **ABSTRACT**

Knowledge of infectious diseases in wildlife provides important information for preventing potential outbreaks of zoonotic diseases. Adiaspiromycosis is a neglected human disease caused by dimorphic Onygenales fungi. The disease is produced by the infammatory response against growing adiaspores, leading to granulomatous pneumonia. In humans, adiaspiromycosis is relevant in immunosuppressed patients. In animals, it is associated with pneumonia in fossorial species. Given the potential role of armadillos in the epidemiology of adiaspiromycosis, in this study, we sought to investigate the occurrence and pathological features of adiaspiromycosis in roadkilled armadillos. In total, 54 armadillo carcasses were suitable for postmortem pathologic examinations between February 2017 and 2020. Adiaspores, associated with granulomatous lesions, were observed in ten six-banded (*Euphractus sexcinctus*) and two southern naked-tailed armadillos (*Cabassous unicinctus*). A previously uncharacterized Onygenales species was molecularly identifed in two *E. sexcinctus*. In summary, herein we report 12 cases of pulmonary adiaspiromycosis (PA) in two species of freeliving armadillos in Brazil. Both, the morphology of the fungus, as well as the histopathological fndings (granulomatous infammatory response to adiaspores) are consistent with PA; however, as the molecular identifcation difers from the reported species, the potential impact of this fungus for human PA is unknown, and we cannot rule out its impact on public health.

4.1.1 **INTRODUCTION**

Surveillance of infectious diseases in wildlife is a valuable tool for the prevention and reduction of human disease outbreaks[1,2]. Armadillos (order Cingulata) play a role in the epidemiological cycle of some zoonoses (e.g., hanseniasis and paracoccidioidomycosis)[3,4]. Despite being protected species, armadillo meat is commonly consumed in several South American countries[5,6]. Direct contact between humans and armadillos could pose a transmission route for several pathogens, thus, health investigations on armadillos may be relevant to public health.

Adiaspiromycosis is a fungal disease caused by the dimorphic fungi *Emmonsia crescens* and *Blastomyces parvus* (formerly known as *Emmonsia parva*) (family Ajellomycetaceae, order Onygenales), considered saprophytes and commonly isolated from the soil[7]. In contrast to other mycoses, once their conidia enter the host, mainly through respiratory via, they become adiaconidia (adiaspores), precluding their replication but increasing their size[8]. The disease is caused by the host´s immune response against the growing adiaspores, leading to the formation of granulomas[9]. The lesions are mainly restricted to the lungs and occasionally regional lymph nodes, although ocular and systemic adiaspiromycosis have also been described[8,10]. These lesions have been reported in humans, wild fossorial mammals (rodents, moles, armadillos), some species of carnivores, deer, horses and anurans ("Supplementary Material").

The first description of the disease was made in wild rodents of the United States in the 1940s[11]. In humans, the first case was reported in France in the 1960s[12]. Adiaspiromycosis was widely studied in the 1970s, mainly in wild small mammals[13]. Nevertheless, reports have been intermittent during the last decades. While human cases are generally associated with immunosuppression, the clinico-pathological features in free-ranging animals remain largely unknown[14,15,16,17,18]. Human pulmonary adiaspiromycosis (PA) is generally an incidental finding in diagnostic images (X-rays and tomography) and can be a differential diagnosis of tuberculosis and/or pulmonary neoplasms; its definitive diagnosis is made by fungal culture and biopsy[19,20,21]. In Brazil, human cases have increased, and adiaspiromycosis is listed as a class II infectious agent (moderate individual risk and limited risk to the community) by the Ministry of Health[22]. In Brazilian animals, *Emmonsia* sp. was detected by PCR in one nine-banded armadillo (*Dasypus novemcinctus*) killed by motor-vehicle collision (MVC) in Botucatu, São Paulo State, however, no anatomopathological data were reported[23]. Adiaspiromycosis has been also described in three other armadillo species: hairy (*Chaetophractus villosus*), pichi (*Zaedyus pichiy*) and seven-banded armadillo (*Dasypus septemcinctus*) from Argentina[24].

Since there are reports of PA in Brazil, and the etiologic agent has already been reported in armadillos (preliminarily by molecular technique)[23], we hypothesized a possible role of armadillos in the epidemiology of PA. Considering that the number of

wild animals killed by MVC in Brazil is remarkable[25], and the potential role of armadillos in the epidemiology of adiaspiromycosis, in this study, we sought to investigate the occurrence and pathological features of adiaspiromycosis in roadkilled armadillos. Here we report 12 cases of PA in two species of wild armadillos of Brazil: the six-banded armadillo (*Euphractus sexcinctus*) and the southern naked-tailed armadillo (*Cabassous unicinctus*).

4.1.2 MATERIAL AND METHODS

Road monitoring

Between February 2017 and February 2020, a periodic road monitoring was carried out to identify the number of armadillos killed by MVC in two states of Brazil, Mato Grosso do Sul (MS) and Sao Paulo (SP) (See 25 for details)[25]. Epidemiological and biological data [species, age class, sex, season (rainy or dry) and coordinates] were recorded from all carcasses. Roadkilled armadillos in good preservation status were selected for investigate the occurrence and pathological features of adiaspiromycosis by anatomopathological study[33].

Pulmonary adiaspiromycosis survey

Representative tissue samples of lungs, trachea and tracheobronchial lymph nodes were collected, and fixed in 10% neutral buffered formalin or frozen at -20 °C or -80°C. These tissues were processed routinely, embedded on paraffin-wax, sectioned at 5 μm-thick and stained with hematoxylin and eosin (H&E) for routine microscopic analysis. Special stains periodic acid-Schiff (PAS), Grocott methenamine silver (GMS), Masson's trichrome (MS) and Warthin–Starry stain (WS) were applied in selected cases to better characterize the histopathologic findings. Microphotographs of the adiaspores were collected and subsequently measured using the Image J software[34].

Molecular analysis

When adiaspores and/or associated lesions were found in the histopathological evaluation, total DNA was extracted from available frozen lung samples (n = 2) using the ZR Fungal/Bacterial DNA Miniprep kit (Zymo, Irvine, CA, USA), according to the manufacturer's instructions. In cases, without available frozen sample (n = 10), DNA extraction of formalin-fixed and paraffin-embedded (FFPE) tissue was performed according to standardized protocols[35]. A panfungal PCR using the primers ITS1-F and ITS-4 at a melting temperature of 55 °C was performed to amplify a 700-bp fragment comprising the 18S rRNA gene, internal transcribed spacer-1 (ITS-1), 5.8S rRNA gene and ITS-2, until the 26S rRNA gene[36]. The variable regions D1 and D2 of the 26S rRNA gene of fungi were amplified by PCR using primers NL1 and NL4[37]. Amplicons of the expected size were purified with Exo-Sap IT (GE Healthcare, Waukesha, WI, USA) or Illustra DNA and Gel Band Purification kit (GE Healthcare, UK, if two bands were present in the agarose gel) and confirmed by direct Sanger sequencing. After a ClustalW alignment in Mega 7.0[38], the obtained sequences (excluding primers) were compared with those available in GenBank using Blast search (http://www.ncbi.nlm.nih.gov/blast). The percentage of nucleotide (nt) identity of the obtained sequences to the closest available on GenBank was calculated based on p-distance ([1 − p-distance] × 100). A 1000 bootstrap maximum likelihood phylogram was constructed with the obtained ITS sequences, those from selected Onygenales species retrieved from GenBank and Aspergillus terreus as outgroup (MH141230).

Ethical standards

This study was carried out in compliance with the System Authorization and Information on Biodiversity (SISBIO) of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) (license number: 58745) and was approved by the Ethics Committee on Animal Use (CEUA) of the School of Veterinary Medicine and Animal Science—University of São Paulo (FMVZ-USP) (protocol number: 7198020317). All animals were dead at the time of necropsy; no animals were euthanized in this study.

4.1.3 **RESULTS**

During the study period, 54 necropsies were performed in four armadillo species: nine-banded armadillo (*Dasypus novemcinctus*, 48.1%; 26/54), six-banded (*E. sexcinctus*, 38.9%; 21/54), southern naked-tailed (*C. unicinctus*, 9.3%; 5/54) and giant-armadillo (*Priodontes maximus*, 3.7%; 2/54). Geographic distribution of all armadillos is shown in Fig. 1.

Figure 1. Geographic distribution of necropsied armadillos in roads of Mato Grosso do Sul and Sao Paulo states (Brazil) between February 2017 and February 2020. The map was created by software QGIS 3.16 (https://qgis.org/).
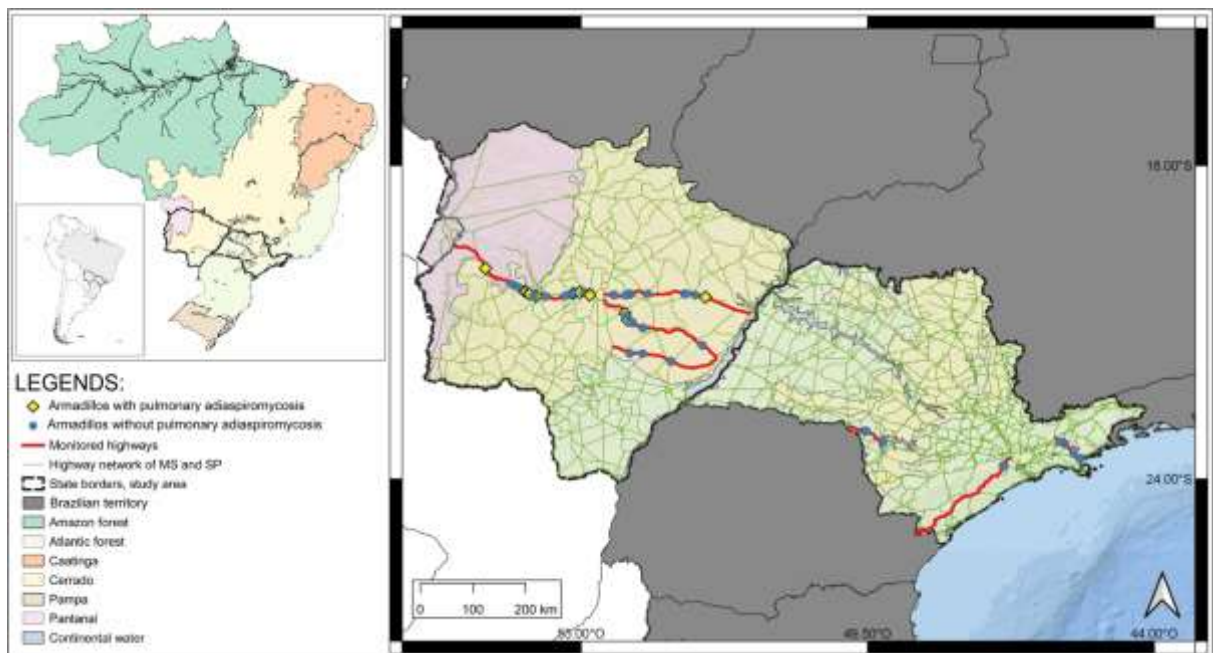
Ten *E. sexcinctus* (47.6%; 10/21) and two *C. unicinctus* (40%; 2/5) showed pulmonary histopathological findings with adiaspores morphologically most compatible with *Emmonsia crescens* or *Blastomyces parvus*. These cases were distributed in seven municipalities of MS, while no cases were found in SP. Complete biological and epidemiological data of armadillos with adiaspiromycosis are listed in Table 1. The main pulmonary gross findings associated with motor vehicle collisions (MVC) were hemorrhage (100%; 12/12), parenchymal rupture (66.7%; 8/12) and congestion (50%; 6/12) ("Supplementary Material"). Additionally, scattered 0.5–1 mm in-diameter,

slightly demarcated, pale tan to yellow nodules were observed throughout the parenchyma of all lung lobes (25%; 3/12), these nodules corresponded to PA (Fig. 2).

Figure 2. (a) Case 10; adult, female *E. sexcinctus*. The left lung lobes have heterogeneous red coloration as well as multifocal, pale tan to white subpleural nodules (yellow arrows). (b) Case 1; adult *E. sexcinctus*. The pulmonary parenchyma has multifocal subpleural and intraparenchymal nodules (yellow arrows) variably associated with bronchi or bronchioles. (c) Case 4; adult, male *Cabassous unicinctus*. Lungs. Note one adiaspores (asterisk) in the bronchial lumen surrounded by inflammatory cells. H&E; bar = 100 µm. (d) Case 10; Adult, female *E. sexcinctus*. Note a granuloma with fragments of the adiaspores wall (asterisk) in the necrotic center. H&E; bar = 200 µm. (e) Case 1; adult *E. sexcinctus*. Note the trilaminar wall of the adiaspores surrounded by few leukocytes. PAS; bar = 50 µm. (f) Case 1; adult E. sexcinctus. Detail of primarily neutrophils targeting the adiaspores wall. H&E; bar = 25 µm. (g) Case 1; adult *E. sexcinctus*. GMS stain highlights the adiaspores wall. Bar = 25 µm.
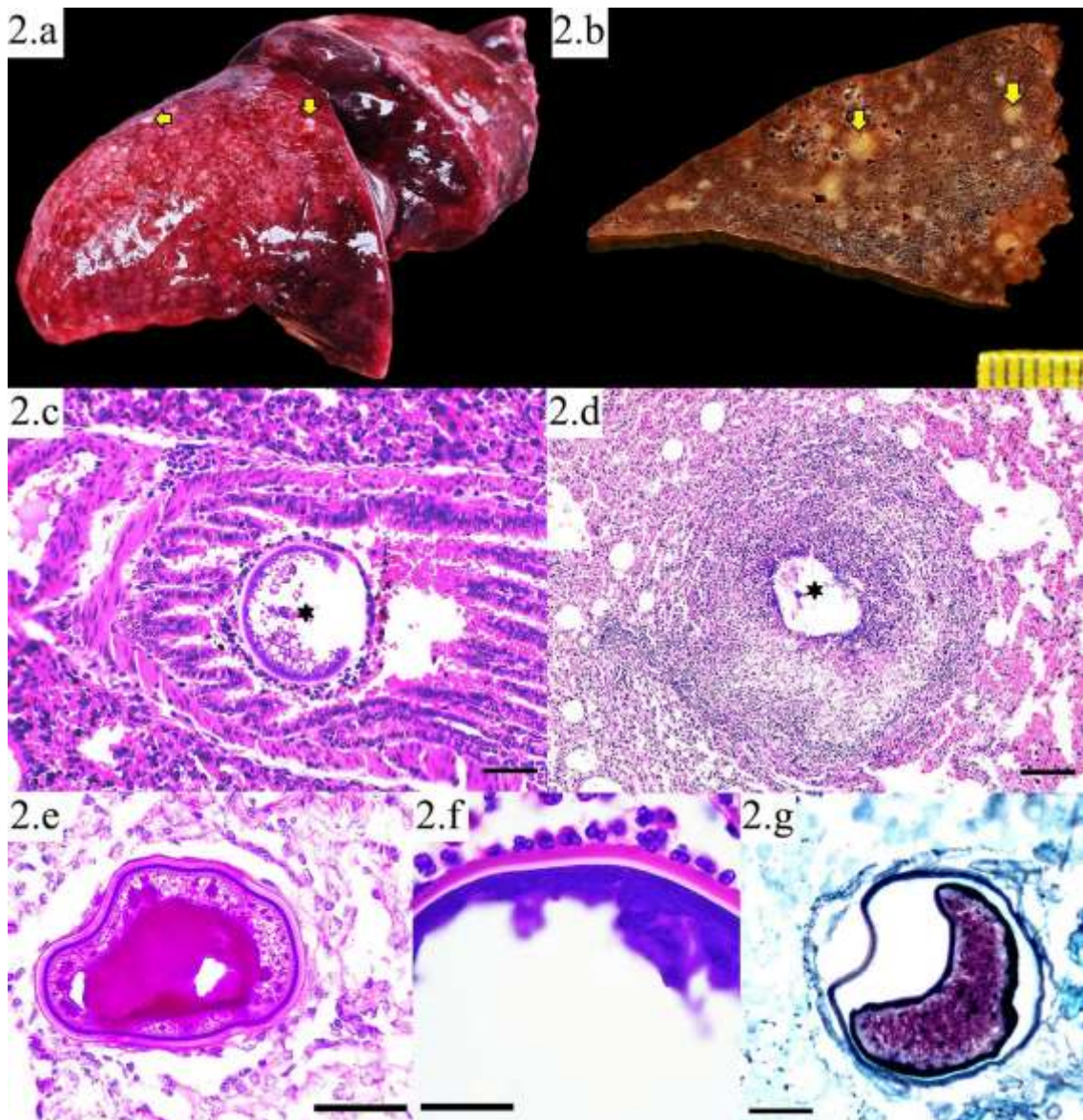
Table 1. Biological and epidemiological data wild armadillos with pulmonary adiaspiromycosis died by motor vehicle collisions in Brazil, 2017–2020.

Case	Common name	Species	Age-class	Sex	Year	Month	Season	Municipality	State
1	SBA	*Euphractus sexcinctus*	Adult	Undetermined	2017	November	Dry	Miranda	MS
2	SBA	*Euphractus sexcinctus*	Adult	Female	2017	March	Dry	Terenos	MS
3	SBA	*Euphractus sexcinctus*	Adult	Female	2017	June	Dry	Terenos	MS
4	SNTA	*Cabassous unicinctus*	Adult	Male	2017	October	Rain	Água Clara	MS
5	SBA	*Euphractus sexcinctus*	Adult	Male	2017	November	Rain	Terenos	MS
6	SNTA	*Cabassous unicinctus*	Juvenile	Male	2017	December	Rain	Campo Grande	MS
7	SBA	*Euphractus sexcinctus*	Adult	Female	2017	December	Rain	Aquidauana	MS
8	SBA	*Euphractus sexcinctus*	Adult	Male	2018	January	Rain	Aquidauana	MS
9	SBA	*Euphractus sexcinctus*	Adult	Male	2018	February	Rain	Anastácio	MS
10	SBA	*Euphractus sexcinctus*	Adult	Female	2018	June	Dry	Anastácio	MS
11	SBA	*Euphractus sexcinctus*	Adult	Male	2018	July	Dry	Terenos	MS
12	SBA	*Euphractus sexcinctus*	Adult	Male	2018	July	Dry	Ribas do Rio Pardo	MS

SBA Six-Banded Armadillo, SNTA Southern Naked-Tailed Armadillo.

Microscopically, all these armadillos had pulmonary adiaspores with varying degrees of localized inflammation, ranging from none to marked granulomatous response (Table 2). Morphologically, adiaspores presented a bi- or trilaminar wall comprising a thin and brightly eosinophilic outer layer, a thick pale eosinophilic mid layer, and an internal basophilic layer which surrounded a core of round basophilic granular material but did not exhibit endospores. Adiaspores averaged 91.1 µm in diameter (ranged from 10.9 to 839.5 µm) and were distributed multifocally within the alveolar and bronchial interstitium spaces. Adiaspores' wall components were highlighted by PAS, GMS and WS stains (Fig. 2). Inflammation severity correlated to adiaspores' size and varied from few reactive macrophages surrounding the adiaspores to severe nodular granulomatous inflammation with large numbers of

epithelioid macrophages, neutrophils, lymphocytes, eosinophils, rare multinucleated giant cells (foreign body type), and fibroplasia. The granulomas could contain a viable adiaspores (up to 200 μm in diameter) or cores with empty adiaspores admixed with neutrophils and necrotic cell debris. Several cases (91.7%, 11/12) had hyperplasia of the lymphoid tissue associated with bronchi and bronchioles. This prompted histochemical investigation for potential *Mycoplasma* sp. and/or *Filobacterium* sp. (formerly CAR Bacillus) structures; however, these were not detected by WS stain. One armadillo had pulmonary coinfection with a trichurid nematode leading to marked bronchitis. Complete microscopic findings are listed in Table 3. No fungal structures and/or associated inflammatory response were observed in other tissues {skin, skeletal muscle, tongue, oropharynx, tonsil, salivary glands, esophagus, stomach, small and large intestines, liver, gallbladder, pancreas, larynx, trachea, lung, heart, great vessels, thymus, spleen, lymph nodes (mandibular, prescapular, mediastinal and mesenteric), kidney, urinary bladder, thyroid and parathyroid glands, adrenal glands, diaphragm, cerebrum, cerebellum, eye, mammary gland, testicle, ovary, uterus, epididymis and prostate} examined.

Table 2. Morphological characteristics of the adiaspores observed in lungs of wild armadillos died by motor vehicle collisions in Brazil, 2017–2020.

Case	Number of adiaspores	Histologic distribution	Diameter (microns)	Min (microns)	Max (microns)	Inflammatory response
1	47	Alveolar, peribronchial	41.9	10.9	177.5	Granuloma
2	2	Peribronchial	66.1	52.3	79.9	Histiocytic infiltration
3	15	Alveolar, peribronchial	114.4	50.6	507.5	Histiocytic infiltration
4	3	Alveolar, peribronchial	292.4	257.7	319.8	Granuloma
5	10	Alveolar, peribronchial	39	16.9	81.5	Granuloma
6	1	Alveolar	277.9	277.9	277.9	Histiocytic infiltration
7	6	Alveolar, peribronchial	36.6	29.5	41.3	Histiocytic infiltration
8	1	Peribronchial	53.3	53.3	53.3	Histiocytic infiltration
9	4	Alveolar, peribronchial	79.5	69.4	94.7	Histiocytic infiltration
10	13	Alveolar, peribronchial	187.8	67.3	839.5	Granuloma
11	4	Peribronchial	95.6	34.8	220.9	Granuloma
12	5	Alveolar, peribronchial	266	65.8	485.8	Granuloma

Two 640-bp sequences (after excluding primers) of the fragment comprised between 18S rRNA gene and 26S rRNA gene were obtained from frozen lung samples of two six-banded armadillos (cases 10 and 12) with pulmonary intralesional adiaspores. The 26S rRNA was also amplified and sequenced in these two animals.

These sequences are available through the GenBank database [MT258564, MT258563, MT258566 and MT258565]. No sequences were recovered from the DNA extractions from FFPE lung samples of the remaining ten armadillos with adiaspores for any of the two selected PCR protocols.

The ITS sequences obtained from two six-banded armadillos (case 10 and 12) were identical between them and had the highest nucleotide (NT) identity (87.6%) to an uncultured organism amplified from the soil of the rhizosphere of tomato (*Solanum lycopersicum*) in Mexico (JN660517), likely *E. crescens* (Cordero-Ramirez et al. 2012), followed by 86.5% similarity with two *E. crescens* sequences from lung samples of mammals (AF038334, AF038337). The unique sequence type found in armadillos clusters separately in our ITS phylogram (Fig. 3).

Table 3. Microscopic pulmonary findings in armadillos with pulmonary adiaspiromycosis died by motor vehicle collisions in Brazil, 2017–2020. [a]Findings associated with PA. BALT bronchi- and bronchiole-associated lymphoid tissue.

Microscopic finding	Total
Vascular/hemodynamic	
Congestion	100% (12/12)
Hemorrhage	100% (12/12)
Edema	83.3% (10/12)
Endothelial hypertrophy	75% (9/12)
Hemosiderosis	58.3% (7/12)
Tunica media hypertrophy/hyperplasia	50% (6/12)
Vasculitis/perivasculitis	50% (6/12)
Alveoli, bronchial/bronchiolar submucosa, interstitium, pleura	
Macrophagic infiltration[a]	100% (12/12)
Presence of adiaspores[a]	100% (12/12)
Lymphocytic infiltration	91.7% (11/12)
BALT hyperplasia	91.7% (11/12)
Hemorrhage	83.3% (10/12)
Atelectasia	83.3% (10/12)
Plasma cell infiltration	66.7% (8/12)

Bronchoconstriction	58.3% (7/12)
Granuloma	50% (6/12)
Anthracosis	50% (6/12)
Neutrophilic infiltration[a]	41.7% (5/12)
Eosinophilic infiltration	41.7% (5/12)
Hemosiderophages	41.7% (5/12)
Multinucleated giant cells	33.3% (4/12)
Nematode eggs (Capillaria sp.)	25% (3/12)
Mucus	25% (3/12)
Fibrin	16.7% (2/12)
Fibrosis	16.7% (2/12)
Necrotic cell debris	8.3% (1/12)
Aspirated particles	8.3% (1/12)
Mucosa/epithelium	
Sloughing/loss	100% (12/12)
Erosion	100% (12/12)
Hyperplasia of goblet cells	50% (6/12)
Bone marrow metaplasia	41.7% (5/12)
Multinucleated giant cells	33.3% (4/12)
Necrosis	25% (3/12)
Type II pneumocyte hyperplasia	25% (3/12)
Calcification	8.3% (1/12)

The two 26S rRNA sequences obtained in this study were highly similar (97.9% nt identity) to Emmonsiellopsis terrestris from the United States (AF038320) and Spain (KP101583), both identified from soil. The former sequence is identified in GenBank as Emmonsiella sp., but after that the same authors reclassified the sequence as Emmonsiellopsis terrestris (Marin-Felix et al. 2015). High similarity (97.6%) was also observed to strains of Paracoccidioides brasiliensis (U93304.1, AF038360.1), and to Emergomyces europaeus (EF592164.1), E`. pasteurianus (EF592152) and different B. parvus sequences (identified in GenBank as E. parva, e.g., AF038331.1, AF038329.1, AF038328.1) with 97.4% identity (Fig. 3).

Figure 3. Maximum likelihood phylogram with 1000 bootstrap replicates of the alignment of the consensus internal transcriber spacer (ITS) 1, 5.8S rRNA gene and ITS 2, nucleotide sequence obtained in two six-banded armadillos (*Euphractus sexcinctus*) from this study, 35 selected fungi of the order Onygenales and *Aspergillus terreus* (MH141230) as outgroup.
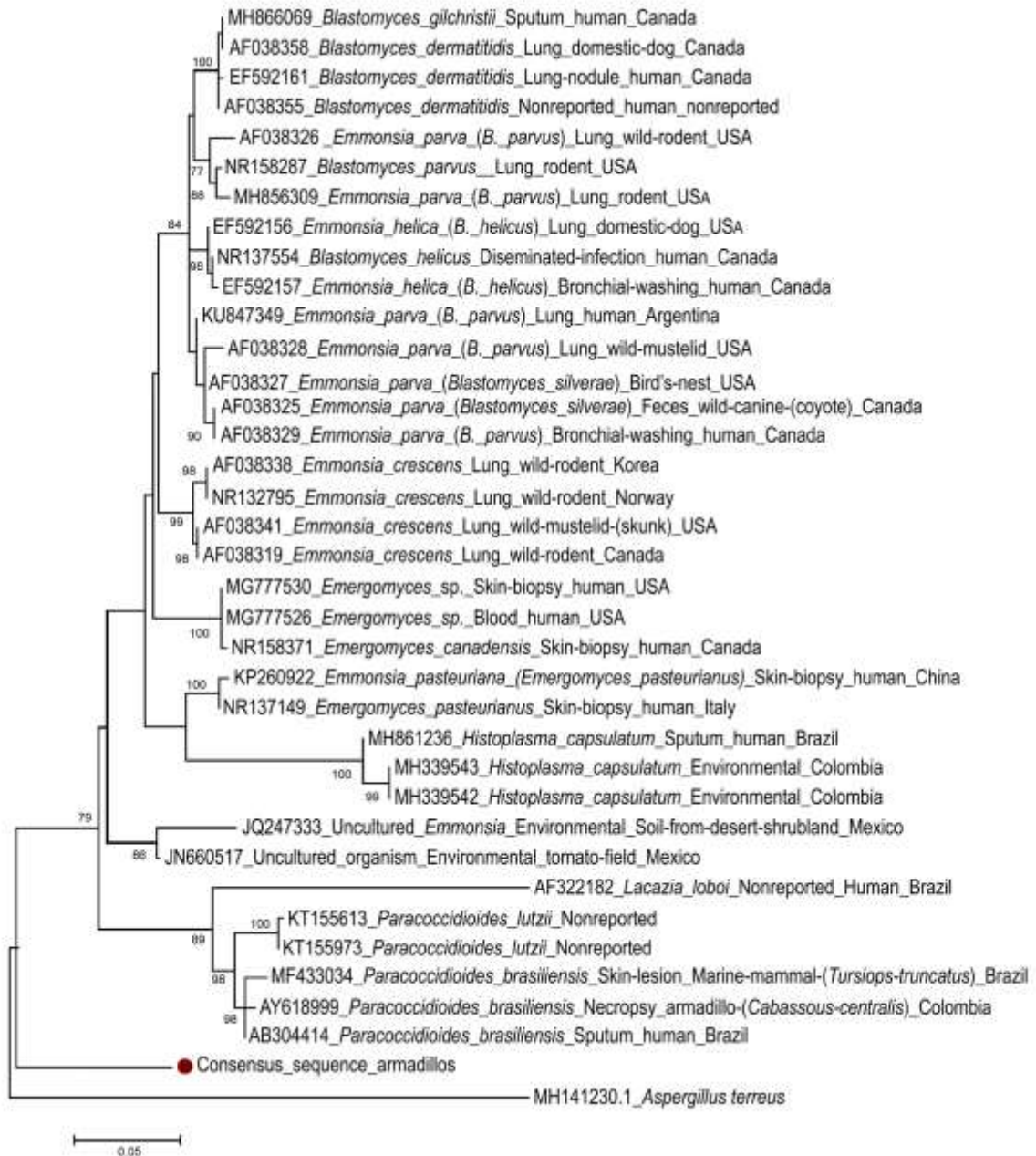
4.1.4 **DISCUSSION**

Armadillos are among the most common species recorded in Brazilian roadkill monitoring programs, in this study were registered a total of 2941 armadillo carcasses over a 3-year period. This is primarily because some species in this group are generalist with large distribution areas and relatively high densities[26,27,28]. Examination of roadkill wildlife is pivotal for a better understanding and monitoring of infectious diseases with zoonotic potential, particularly in synanthropic and peri-urban wild animals[1,29]. In this study, armadillos killed by MVC were regarded as an important source of information for the surveillance of PA.

Adiaspiromycosis and emmonsiosis-like fungi are isolated from the soil[7]. Previous studies detailed that this order (Cingulata) presents a higher occurrence of infection by Emmonsia adiaspores when compared to other mammals (marsupials, rodents, lagomorphs and carnivores)[13]. It is reasonable to believe that the fossorial and digging habits of armadillos likely promote inhalation of soil adiaspores and subsequent lung lesions. However, we only found cases in two of the four species collected. However, the habits of *D. novemcintus* are less fossorial than *E. sexcinctus* and *C. unicinctus* is actually considered subterranean[30]. The low sample size of *P. maximus* (N = 2) may be why no cases were detected.

Microscopically, PA in man and animals is characterized by granulomatous pneumonia with intralesional adiaspores. The local inflammatory response includes histiocytes, neutrophils, epithelioid macrophages, lymphocytes, and occasionally multinucleated giant cells; and this is directly proportional to the adiaspores size. Adiaspores are usually bi- to trilaminar and their wall stains positive by PAS and GMS. *E. crescens* has a larger diameter (200–700 µm) compared to *B. parvus* (20–40 µm)[13,16,31]. These characteristics were observed in the armadillos analyzed in this study, however, even though the diameter of the adiaspores averaged 129.2 µm (Min = 10.9 µm; Max = 839.5 µm), being compatible with *E. crescens*, only 86.5% nt similarity for ITS region was obtained. The culture of these fungi was not performed due to their difficulty and the need of high biosecurity levels, fact that limited technically the morphologic characterization of the fungi[31].

The identities of our ITS sequences with the nearest ones is even lower than those reported for core genes of *E. parva* isolates (88.6%), a species proposed as polyphyletic, and that those proposed for *E. crescens* strains (91.8%) based on genome identity[32]. The D1-D2 sequences are highly similar to other Onygenales species. Based on the molecular results, we identified a hitherto undescribed Onygenales species in pulmonary lesions of two six-banded armadillos causing adiaspiromycosis. Interestingly, aside from *E. crescens* and *B. parvus*, potential novel fungal species have been described in wild mammals with adiaspiromycosis, including the report of a unique sequence type more related to *Emmonsiellopsis* in two northern hairy-nosed wombats (*Lasiorhinus krefftii*) a fossorial species from Australia[13]. The zoonotic potential of the fungus infecting armadillos is unknown. Our molecular results raised the question about which adiaspore-producing species are infecting humans in Brazil, in light of most of the human cases are diagnosed only based on morphology, without ancillary molecular testing. To this date, in a literature review, at least 13 reports of human PA are available in the country ("Supplementary Material"). Furthermore, superficial adiaspiromycosis was reported to cause granulomatous conjunctivitis in children in the Amazon basin, and histopathologic examination of ocular nodules identified adiaspores-like structures[10]. Future studies molecularly identifying the fungal species in humans are necessary to explore the epidemiology of the disease, including the use of armadillo species as indicator of potential exposure areas to humans.

Data availability

All data generated or analyzed during this study are included in this published article.

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4.2 EXPOSURE TO CARBAMATE AND ORGANOPHOSPHATE PESTICIDES IN BRAZILIAN WILD CARNIVORES: CONTRIBUTIONS FROM ROAD MORTALITY.

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4.2.1 **ABSTRACT**

The populations of land carnivores worldwide are suffering sizeable threats to their conservation. Within these, the anthropic processes that induce an increase in unnatural mortality like poisoning by indiscriminate pesticide use and Motor-Vehicle Collisions (MVC) are highly impactful. This study aimed to perform a toxicological screening to detect three pesticides in wild carnivores from road mortality in São Paulo State, Brazil. Two carbamates were selected: aldicarb, carbofuran, and one organophosphate: phorate. The biological matrices selected were liver and vitreous humor. Overall, 35 wild carnivore carcasses were studied, including felids, canids, mustelids, and procyonids. The presence of at least one pesticide occurred in 34.3% (12/35) of the cases. Aldicarb was present in nine cases, carbamate in two, and phorate in one case. Our results do not define the source of exposure, the route of ingestion, or direct poisoning. However, since it is well established that poisonings by the groups of pesticides studied induce neurological effects, we cannot rule out that the presence of pesticides could favor MVC in these carnivores. In addition, the pesticides studied were selected for being frequent in wildlife poisonings across the world and for being currently banned in Brazil; therefore, its detection indicates failures in the inspection and control strategies.

Keywords: Atlantic Forest, human-carnivore conflict, forensic toxicology, toxicology, wildlife roadkill.

4.2.2 **INTRODUCTION**

Brazil has 28 species of terrestrial carnivore mammals distributed in its territory (ICMBio 2021). Its presence in the country is a good indicator of healthy ecosystems. However, habitat loss and fragmentation, unnatural mortality from direct hunting, Motor-Vehicle Collisions (MVC), and direct poisoning are the main threats to its conservation (del Rio et al. 2001, Karanth & Chellam, 2009; Treves, 2009). In Sao Paulo, a recent study showed that 37,744 mammals died of MVC between 2005-2014,

and wild carnivores represented the second most affected group with 24.5% of mortality cases (Abra et al. 2021).

Brazil is one of the top world producers and exporters of agricultural commodities as sugar cane, coffee, soybean, meat, and ethanol. The Brazilian agribusiness is one of the chief economic pillars, participating in more than 20% of the Gross Domestic Product (GDP) (CEPEA-USP, 2019). In addition, the Brazilian territory hosts more than 120 thousand wild species and has the main number of endemic species worldwide (ICMBio, 2021). However, due to the necessary transformation of land use for agricultural plantations or cattle grazing, the relationships between agriculture and biodiversity have historically conflicted. The One Health approach indicates that we are all part of the same territory, and environmental threats can affect humans and animals. That said, current challenges require coexistence and synergism between agricultural production and biodiversity conservation (WHO, 2016).

Pesticides are one of the main strategies to maintain food production, seeking to guarantee its supply worldwide. However, the indiscriminate and inappropriate use of these substances causes damage to the ecosystem, human and animal health (Popp, 2013; Kim, 2016). Official reports indicate that, in 2017, Brazil used more than 530 thousand tons of pesticides, making it one of the world's head consumers (Pignati et al. 2017). In addition, in Brazilian studies, it has been documented that the rural population is vulnerable to the adverse effects and even poisoned by the use of these substances (Nerilo et al. 2014; Marcelino et al. 2019; de Moraes Filho et al. 2020). Worldwide there is information about mortality outbreaks in wildlife in plantations where agrochemicals were used or even direct poisoning (Ruiz-Suárez et al. 2015). In recent years two Brazilian studies were published indicating the presence of pesticides in lowland tapirs (Medici et al. 2021) and hyacinth macaws (Vicente & Guedes 2021). However, studies assessing the direct and indirect effects of the excessive use of pesticides on Brazilian wildlife are still scarce.

Carbamates (CB) and organophosphates (OP) are the most common pesticides reported to be found in wildlife toxicological studies (Ogada 2014; Ruiz-Suárez et al. 2015). Their compounds are exclusive for agricultural use, and their mechanism of action is the inhibition of the enzyme acetylcholinesterase (Risher et al. 1987). In

mammals, CB and OP absorption are mainly through the gastrointestinal and respiratory tracts. However, there are reports of dermal absorption. Metabolization occurs rapidly in the liver, although it can also be found in the blood and other matrices such as the vitreous humor (de Siqueira et al. 2015; Spinosa et al. 2017). Excretion is urinary and occurs quickly during the first 24 hours, and complete elimination can last five days. No bioaccumulative effect has been determined even after continuous exposure (Risher et al. 1987). Toxicological screening must be carried out to detect both compounds and their metabolites since metabolites can have even more toxic effects. For example, aldicarb (AD) has two metabolites, aldicarb sulfoxide (ASX) and aldicarb sulfone (ASN), which have 23 times and 60 times more toxic effects than AD, respectively (Montesissa et al. 1994). Toxic effects include vomiting, abdominal pain, lacrimation, bradycardia, tremors, seizures, and respiratory insufficiency with death from asphyxia (Risher et al. 1987; Spinosa et al. 2017).

Based on the facts that Brazil has a great diversity of wild carnivores, with 18 species distributed in the state of São Paulo, in São Paulo State, the transformation of land use has been significant, and available data indicate that carnivores are a group frequently impacted on road mortality, we formulate two assumptions: a) the use of road mortality can be a source of information to identify pesticides in wildlife and prioritize study areas; b) pesticide poisoning can favor MVC since these substances can generate neurological alterations. Consequently, this study aimed to perform a toxicological screening to detect three pesticides in wild carnivores from road mortality in the state of São Paulo. Two carbamates were selected: aldicarb (AD), carbofuran (CB), and one organophosphate: phorate (PH). The biological matrices selected were liver and vitreous humor.

4.2.3 **MATERIAL AND METHODS**

Study area

The state of São Paulo is located in the Brazilian southeast. It is the most populated state in the country and contributes about 34% of the national GDP. It has a tropical climate with seasonal differences in temperature (22–28 ºC) and precipitation

(1450–2050 mm per year). Its road system is one of the largest in the country, exceeding 199,371 km, although more than 80% are unpaved roads (DER, 2019; IBGE, 2021; INMET, 2021). The Atlantic Forest was the biome that covered in the past more than two parts of the State land territory. Due to the transformation of land use by agricultural activities and urban growth, currently, there are few fragments of primary forests. It is noted that this biome has the highest number of extinct or endangered species and is an important focus for biodiversity (SOS Mata Atlântica, 2014).

According to physical, economic, social, and human characteristics, the state of São Paulo has 15 mesoregions (IBGE). This study selected roads in three mesoregions: Assis, Litoral Sul, and Vale do Paraíba Paulista (Fig 1).

Assis mesoregion has 35 municipalities, has an area of 12,702 km², and a population density of 47.2 inhabitants/km². In 2016 its participation in the agricultural sector of the state was 4.83% (SDE, 2019). The selected road was a 50 km stretch that passes through the cities: Pirajú, Bernardino de Campos, Ipaussu, Chavantes, Canitar, and Ourinhos. The road is a single line with an average speed of 80 km/h. The average daily volume (ADV) of vehicles varies from 5.1-5.9 vehicles/day.

Litoral Sul has 17 municipalities, has an area of 13,185km², and a population density of 38.5 inhabitants/km². In 2016 its participation in the agribusiness of the state was 3.7% (SDE 2019). In this region is placed the Ribeira Valley. Its main economic activities are agriculture, mining, and plant extraction. The selected road has 402 km and connects the cities of Sao Paulo (capital of SP) and Curitiba (capital of Paraná State). This road is entirely two-lane and has a speed limit of 110km/h for cars and 80 km/h for trucks.

Paraíba Paulista Valley has 39 municipalities, has an area of 16,181km², and a population density of 157.8 inhabitants/km². In 2016 its participation in the agribusiness of the state was 1.24 (SDE 2019). The landscape is mainly grassland, eucalyptus plantations, and urban areas. However, in this mesoregion, there is also one of the main preservation areas of Mata Atlantica, the Serra do Mar State Park. The selected road has 82 km and connects the cities of São José dos Campos (on the continental plateau) with Caraguatatuba (on the Atlantic coast). It is a mixed road with sections of

a single line (mainly in the Serra do Mar National Park) and transects with two-lane with New Jersey separators.

The study

Two strategies were designed and implemented to obtain the carcasses of wild carnivores:

a) monitoring of toll roads: Brazilian legislation (federal and state) requires toll road companies to formulate and implement an environmental plan. In addition, this plan should contain strategies to improve wildlife communication (underpasses, overpasses, and canopy). And a road mortality monitoring program shall be implemented. During the monitoring by traffic inspectors, if a domestic or wild animal is on the road (live or death), it must be removed immediately to avoid accidents. In the agreement, traffic inspectors should collect the carcasses of wild carnivores in plastic bags (biohazard). The specimens were preserved in a freezer in the operational control center (OCC) until transport to the laboratory.

b) Passive collection of corpses by public or private institutes: as a strategy to increase the representativeness of this study, we made agreements with entities specialized in wild carnivores since the facilities usually have freezers with specimens killed by MVC.

The partnership lasted 24 months (starting in January 2017). It was also collected the geographic coordinates and the date of death. The authors carried out the identification of the species based on morphological characteristics. The Department of Pathology of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo (VPT-FMVZ-USP) carried out the pathological analysis. And the Laboratory of Pharmacology and Toxicology of the same institution (LADTOX, VPT-FMVZ-USP) carried out the toxicological analysis. The transportation of the carcasses was carried out every six months. For each case, we recorded the date of death, the geographic coordinates, the municipality, the road, the sex, the age class, and the nutritional status (poor, regular, good, obese).

The Ethics Committee on Animal Use on Research of the FMVZ/USP approved this study under protocol number: 7198020317, and the System Authorization and Information on Biodiversity (SISBIO) of the Chico Mendes Institute of Biodiversity Conservation (ICMBio) approved this study with the license number: 27587-12.

2.3 Sample collection and toxicologic study

For the collection of the samples the protocols proposed by King (2014) were adopted. For the collection of liver tissue, approximately 50 grams were collected, including fragments of the left and right lobes. For the vitreous humor, we used a sterile 10 mL syringe with a hypodermic needle of 18G*70mm (for medium and large animals, <5kg) and 21G*50mm (for small animals, > 5kg). We used one thumb tissue forceps to hold the eye. Subsequently, the needle was inserted through the lateral edge at an angle of approximately 90°. Then, the needle was introduced approximately 2cm (with negative pressure) to obtain between 0.5 ml and 3 ml of vitreous humor (from each eye). All samples were collected in sterile 15- or 50-mL Falcon centrifuge tubes and maintained at -20° C until the toxicological analysis. It was not possible to perform a systematic collection of the two samples in all cases due to the injuries caused by CVS (for example, ocular rupture or evisceration).

The detection and identification of pesticides were carried out using the method of High-Performance Liquid Chromatography with diode-array detector (HPLC-DAD) following standardized protocols in the LADTOX (de Siqueira et al. 2015; Gonçalves-Júnior et al. 2017). In this screening two carbamate pesticides (CB) were studied: aldicarb (AD) and its metabolites (aldicarb-sulfoxide and aldicarb-sulfone), carbofuran (CB) and its metabolite (3-OH-carbofuran). As well as one organophosphate (OP): phorate (PH) and its metabolite (phorate-sulfoxide).

4.2.4 **RESULTS**

Overall, 35 specimens were collected (Figure 1). According to the IUCN Red List, this study includes one vulnerable (V) species, two near threatened (NT), and six species in the category least concern (LC). According to the ICMBio Red Book

(ICMBio, 2021), there are four V species, one NT, and four LC species. In table 1 were recorded detailed information of all cases.

Figure 1. Spatial distribution of wild carnivores included in this study, red circle positive animals, blue point negative individuals.
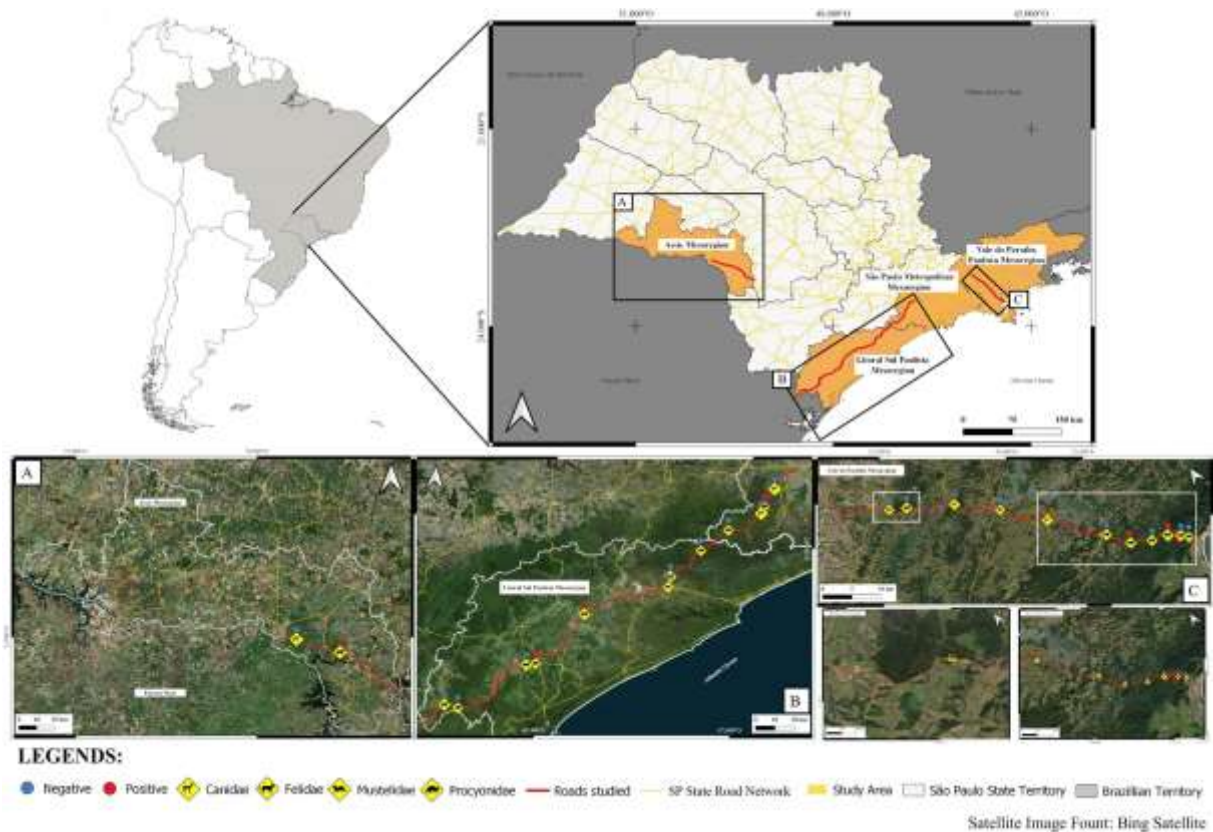
The presence of at least one pesticide occurred in 34.3% (12/35) of the cases. In nine animals, AD or its metabolites were detected. These cases comprise two maned wolves (*Chrysocyon brachyurus*), two jaguarundis (*Puma yagouaroundi*), two pumas (*Puma concolor*), one ocelot (*Leopardus pardalis*), one crab-eating raccoon (*Procyon cancrivorus*), and one Lesser Grison (*Galictis cuja*). CB or its metabolites was detected in two animals, one ocelot (*L. pardalis*) and one crab-eating raccoon (*P. cancrivorus*). Finally, PH was detected only in one jaguarundi (*P. yagouaroundi*). In table 2 were recorded detailed information on pesticides and biological matrices. The two biological matrices (liver, vitreous humor) presented results for the three pesticides studied.

Table 1. General data of 35 wild carnivores killed by motor vehicle-collision (MVC) included in this study

Species	Sex		Age		Body condition		Season		Presence of Pesticides
	Female	Male	Juvenile	Adult	Regular	Good	Dry	Rain	
Crab-eating Fox (C*erdocyon thous*) n=4	50% (2/4)	50% (2/4)	0% (0/4)	100% (4/4)	0% (0/4)	100% (4/4)	100% (4/4)	0% (0/4)	0% (0/4)
Crab-eating Raccoon (*Procyon cancrivorus*) n=7	28.6% (2/7)	71.4% (5/7)	42.9% (3/7)	57.1% (4/7)	0% 90/7)	100% (7/7)	14.3% (1/7)	85.7% (6/7)	28.6% (2/7)
Jaguarundi (*Herpailurus yagouaroundi*) n=4	0% (0/4)	100% (4/4)	50% (2/4)	50% (2/4)	25% (1/4)	75% (3/4)	0% (0/4)	100% (4/4)	75% (3/4)
Lesser Grison (*Galictis cuja*) n=3	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)	33.3% (1/3)	66.7% (2/3)	33.3% (1/3)	66.7% (2/3)	33.3% (1/3)
Maned Wolf (*Chrysocyon brachyurus*) n=4	50% (2/4)	50% (2/4)	25% (1/4)	75% (3/4)	25% (1/4)	75% (3/4)	25% (1/4)	75% (3/4)	50% (2/4)
Neotropical Otter (*Lontra longicaudis*) n=1	0% (0/1)	100% (1/1)	0% (0/1)	100% (1/1)	0% (0/1)	100% (1/1)	100% (1/1)	0% (0/1)	0% (0/1)
Ocelot (*Leopardus pardalis*) n=7	28.6% (2/7)	71.4% (5/7)	42.9% (3/7)	57.1% (4/7)	14.3% (1/7)	85.7% (6/7)	42.9% (3/7)	57.1% (4/7)	28.6% (2/7)
Puma (*Puma concolor*) n=4	50% (2/4)	50% (2/4)	100% (4/4)	0% (0/4)	25% (1/4)	75% (3/4)	50% (2/4)	50% (2/4)	50% (2/4)
Southern Tiger Cat (*Leopardus guttulus*) n=1	100% (1/1)	0% (0/1)	100% (1/1)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/1)	100% (1/1)	0% (0/1)
Total	31.4% (11/35)	68.6% (24/35)	40% (14/35)	60% (21/35)	14.3% (5/35)	85.7% (30/35)	37.1% (13/22)	62.9% (22/35)	34.3% (12/35)

Table 2. General data and pesticide product observed in 12 wild carnivores killed by Motor Vehicle-Collision (MVC) included in this study

Case	Species	Sex	Age	A	ASX	ASF	C	3OHC	PH
C-1	*Chrysocyon brachyurus*	Female	Adult	L	-	-	-	-	-
C-2	*Chrysocyon brachyurus*	Male	Juvenile	VH	VH	VH	-	-	-
C-3	*Galictis cuja*	Male	Adult	L	-	-	-	-	-
C-4	*Herpailurus yagouaroundi*	Male	Adult	L	-	-	-	-	-
C-5	*Herpailurus yagouaroundi*	Male	Juvenile	L	-	-	-	-	-
C-6	*Herpailurus yagouaroundi*	Male	Adult	-	-	-	-	-	L+VH
C-7	*Leopardus pardalis*	Male	Adult	VH	-	-	-	-	-
C-8	*Leopardus pardalis*	Male	Juvenile	-	-	-	L+VH	L+VH	-
C-9	*Procyon cancrivorus*	Male	Adult	-	-	-	L	L	-
C-10	*Procyon cancrivorus*	Female	Juvenile	L	-	-	-	-	-
C-11	*Puma concolor*	Female	Juvenile	L	L	L	-	-	-
C-12	*Puma concolor*	Female	Juvenile	L+VH	L+VH	-	-	-	-

A=aldicarb; ASF=Aldicarb-sulfoxide; ASF=aldicarb-sulfone; C=carbofuran; 3OHC=3-OH-carbofuran; PH=phorate; L=liver; VH=Vitreous humor

4.2.5 **DISCUSSION**

We report a 34.3% prevalence of pesticides, namely AD, CB, and PH, in the wild carnivores studied. Through toxicological screening, all three pesticides as well

their respective metabolites were identified in liver and vitreous humor samples. Toxicokinetic and toxicodynamic studies indicate that all three pesticides have rapid absorption via the GI tract, followed by fast hepatic metabolism (de Siqueira et al., 2015; Spinosa et al., 2017). Vitreous humor (VH) is a matrix that suffers less from the effects of autolysis. Similarly, some CB and OP pesticides may be present in this fluid. Additionally, HV is considered a top sample in forensic sciences (de Siqueira et al. 2016; Bévalot et al. 2016). Interestingly, these three compounds were banned in Brazil in the last ten years: AD (2012), PH (2014), and CB (2017). Our results reveal the use of banned pesticides in three mesoregions of the state of Sao Paulo. Also, corroborate the efficiency of liver and HV as a helpful sample for the toxicological screening of AD, CB, and PH in wild carnivores.

Here we detect three pesticides in six species of wild carnivores. Reports of the exposure of pesticides in wildlife are relatively common: studies in Spain (75%, 168/225), in South Africa between 2009-2014 were diagnosed 135 cases of poisoning with OP and CB pesticides in South Africa, and in Kenya carbofuran there is report in intentional poisoning of lions and is known as "Lion Killer" (Ogada 2014; Botha 2015; Ruiz-Suárez et al. 2015). Our results demonstrate the use of these pesticides in Brazil. However, we were not able to define if pesticides were used for direct poisoning or indiscriminate use in plantations.

In one of the jaguarundis, the remains of a domestic bird were observed. Habitat loss and change in land use increase the contact between wild species, production/domestic animals, and humans; specifically, in wild carnivores, it is known that intentional poisoning can be a consequence of human-wildlife conflicts (Mateo-Tomás, 2012). Studies in the Atlantic Forest have identified a low tolerance of humans to wild carnivores due to economic losses related to livestock predation (Teixeira et al., 2020). Although the carnivore species studied are perceived as a source of damage by some farmers, our results do not allow us to assure that these animals were intentionally poisoned.

Secondary poisoning may also occur by the incorrect and illegal use of pesticides as poisons for pest control, by accidental ingestion, or by contact with contaminated soil and water (Balcomb, 1983; Elliott et al., 1996; Wobeser 2004). In

Brazil, food poisoning has been reported in humans and wildlife by AD and PH (Bucaretchi et al., 2012; de Siqueira et al. 2016). Therefore, we cannot rule out that the positive animals in this study could have indirect contact with these pesticides.

Our results reveal AD as the most frequent pesticide, suggesting greater use of this substance compared to the other two. In Brazil, AD is associated with "chumbinho" (the common name of the most popular illegal rodenticide). However, since October 2012, the Brazilian Health Surveillance Agency (ANVISA) has banned its use in Brazil (Bucaretchi et al., 2012; de Siqueira et al. 2015). The presence of this pesticide in wild carnivores could be related to its illegal use as a rodenticide. In addition, some carnivores studied here were found close to peri-urban and rural areas, which supports the hypothesis of potential exposure to chumbinho.

OP and CB can affect metabolic functions such as thermoregulation, water/food intake, and behavior patterns, leading to weight loss, development, and reproduction problems (Noyes et al. 2009). Considering that Brazil is one of the largest consumers of pesticides worldwide, more research on ecotoxicology is urgently needed to investigate the effects of pesticides on wildlife. In addition, control of the use and trade of pesticides must be intensified, as well as more research on the exposure of humans and animals to these substances, mainly because Brazil now has more than 493 pesticide compounds approved for use (MAPA, 2021), with unknown consequences for wildlife. It is also necessary to educate people about its use, warning them about the adverse effects and the possible impacts on human and environmental health.

Injuries by MVC were the cause of death in all cases studied. CB and OP have as a mechanism of action the inhibition of the enzyme acetylcholinesterase; muscarinic and nicotinic effects include neurological disturbances (Carlson 1988). Additionally, studies estimate that more than 39 thousand medium and large wild animals die on roads in the state of Sao Paulo each year, with carnivores being the second most frequent order, only surpassed by rodents (Abra et al., 2021). Despite not having been possible to measure the inhibition of acetylcholinesterase, we cannot rule out that these animals had nervous disturbances that could favor the MVC. Our study details two direct impacts on the conservation of wild carnivores in the state of Sao Paulo: roadkill and contact with pesticides. Scientists and decision-makers should encourage

to correlate studies to elucidate the impacts of these factors on the conservation of carnivore populations in Sao Paulo.

4.2.6 **CONCLUSIONS**

The presence of pesticides currently banned in Brazil in more than 30% of the carnivores studied here indicates failures in the inspection and control of these substances in Sao Paulo State. Unfortunately, we were unable to define the pesticide exposure route in the studied carnivores. However, road mortality as a passive fount of samples confirmed the circulation of highly toxic substances in three regions of Sao Paulo State.

Our study highlights the importance of toxicological screenings to contribute to understanding the effect of these compounds in wildlife. Brazil needs to invest more in research on the circulation of currently prohibited and permitted pesticides, seeking to understand the possible impacts on humans, animals, and environmental health, under a One Health perspective.

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5   FINAL CONSIDERATIONS

To summarize, highways have played a fundamental role in developing land transport in Brazil. On the other hand, environmental regulations are relatively recent and are still not applied to all roads today. This author considers that the advances made in environmental policies are partly due to the contribution of academia on how we understand and mitigate the impacts of roads on biodiversity. However, fundamental reforms are still needed to improve the quality and heterogeneity of Brazilian land transport.

In summary, through the systematic pathological study of road-killed wild mammals, various novel pathological findings or disease processes were observed. The presence results highlight the richness of wildlife health information obtained through the study of carcasses of road-kills. These cadavers can be useful to understand the health of different species in a regional and/or local environment. This author strongly recommends this type of study specially in taxa where health information is scarce.

The pathological information obtained through this study has revealed the diversity of infectious and noninfectious processes and/or etiologies to which wild mammal populations in the Cerrado, Pantanal and Mata Atlantica are exposed. Similar studies can be used in epidemiological surveillance programs for livestock and zoonotic diseases as well as for environmental contamination.

In addition, this thesis identified various novel pathological findings in several species. Although the etiopathogenesis was not readily evident for some of them, parasitic infections appear to be prevalent. Moreover, we provide baseline pathology knowledge of wild mammals in South America, specifically in the Brazilian Cerrado, Pantanal and Mata Atlantica.

This thesis highlights the importance of toxicological screenings to contribute to understanding the effect of these compounds in wildlife. Brazil needs to invest more in research on the circulation of currently prohibited and permitted pesticides, seeking to understand the possible impacts on humans, animals, and environmental health, under a One Health perspective.

This author considers that virtually any wild mammal species can be affected by MVC. In addition, the current results suggest that the animals studied here show a good general condition. Just a reduced number shows evidence of preexisting diseases. The preceding assumes that biodiversity loss by MVC can considerably impact a population since the compromised individuals could play a fundamental role in reproductive times. However, more studies are needed to confirm this postulate.

This author believes that the results presented in chapter III compose a solid knowledge base that demonstrates the importance of using road fatality animals in infectious and noninfectious surveillance studies or programs. It must recognize that many species of neotropical biodiversity have health gaps; therefore, the tremendous impact of vehicular collisions in terms of the number of individuals is an opportunity that should not be underestimated.

These results may aid clinicians performing emergency care in MVC patients and may be of value in pathologic and forensic investigations wherein MVC is deemed a likely contributor factor of death. In addition, the data may be helpful to roadkill first responders, caregivers, veterinarians, and diagnosticians.

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APPENDIX I

General information, gross and histopathological in artiodactyls.

Table 1. General information of deer.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-294	*Blastocerus dichotomus*	Male	Adult	-	Good	2018	November	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-001	*Mazama gouazoubira*	Male	Adult	16.5	Good	2016	July	Dry	SP	Juquitiba	Atlantic forest
RK-002	*Mazama gouazoubira*	Male	Adult	15.5	Regular	2016	March	Rain	SP	Registro	Atlantic forest
RK-008	*Mazama gouazoubira*	Female	Adult	20.0	Good	2015	February	Rain	SP	São Lourenço da Serra	Atlantic forest
RK-016	*Mazama gouazoubira*	Female	Juvenile	10.5	Good	2015	June	Dry	SP	Miracatu	Atlantic forest
RK-050	*Mazama gouazoubira*	Male	Adult	17	Good	2016	August	Dry	SP	São Lourenço da Serra	Atlantic forest
RK-082	*Mazama gouazoubira*	Female	Adult	14.5	Good	2017	August	Dry	SP	Miracatu	Atlantic forest
RK-084	*Mazama gouazoubira*	Female	Adult	13.3	Good	2017	October	Rain	SP	Embu	Atlantic forest
RK-086	*Mazama gouazoubira*	Female	Adult	12.2	Good	2017	July	Dry	SP	São Lourenço da Serra	Atlantic forest
RK-172	*Mazama gouazoubira*	Female	Juvenile	10.5	Good	2014	July	Dry	SP	Registro	Atlantic forest
RK-173	*Mazama gouazoubira*	Female	Juvenile	6.9	Regular	2014	July	Dry	SP	São Lourenço da Serra	Atlantic forest
RK-207	*Mazama gouazoubira*	Male	Adult	13.6	Good	2018	May	Dry	SP	Miracatu	Atlantic forest
RK-268	*Mazama gouazoubira*	Female	Adult	16.2	Good	2018	April	Dry	SP	Cajati	Atlantic forest
RK-269	*Mazama gouazoubira*	Female	Adult	18.5	Good	2018	August	Dry	SP	Miracatu	Atlantic forest
RK-270	*Mazama gouazoubira*	Male	Adult	17.9	Good	2018	July	Dry	SP	Cajati	Atlantic forest
RK-271	*Mazama gouazoubira*	Female	Juvenile	10.2	Regular	2018	July	Dry	SP	São Lourenço da Serra	Atlantic forest
RK-281	*Mazama gouazoubira*	Female	Adult	12.6	Good	2018	November	Rain	SP	Juquitiba	Atlantic forest
RK-311	*Mazama gouazoubira*	Female	Adult	10.5	Good	2018	July	Dry	PR	Campina Grande do Sul	Atlantic forest
RK-334	*Mazama gouazoubira*	Male	Adult	21	Good	2018	December	Rain	SC	Itaiópolis	Atlantic forest
RK-336	*Mazama gouazoubira*	Female	Adult	13.21	Good	2018	September	Dry	PR	Rio Negro	Atlantic forest
RK-348	*Mazama gouazoubira*	Male	Adult	19.3	Good	2017	August	Dry	SC	Ponte Alto do Norte	Atlantic forest
RK-385	*Mazama gouazoubira*	Female	Adult	21	Good	2019	April	Dry	SC	Itaiópolis	Atlantic forest
RK-389	*Mazama gouazoubira*	Female	Adult	14.6	Good	2017	July	Dry	SC	Correia Pinto	Atlantic forest
RK-438	*Mazama gouazoubira*	Female	Adult	-	Good	2019	December	Rain	MS	Aquidauana	Pantanal

Figure 1. Spatial distribution, and main characteristics of deer.
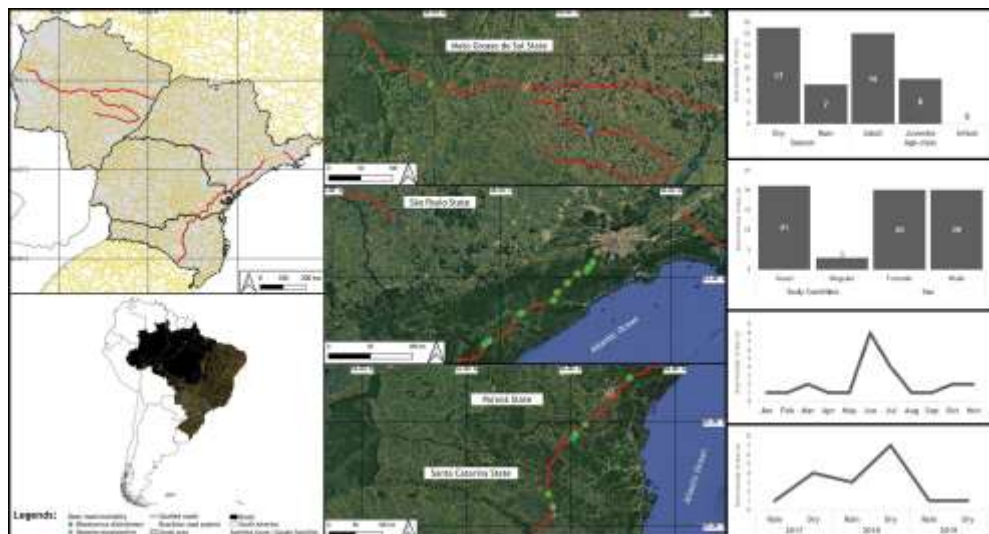
Table 2. Gross findings non-associated with trauma in deer.

Gross finding	Cases
Tick infestation	55% (12/22)
Hydatid, cyst	18% (4/22)
White pulp hyperplasia, spleen	14% (3/22)
Splenomegaly	14% (3/22)
Pregnancy	9% (2/22)

Figure 2. Main Gross findings in artiodactils
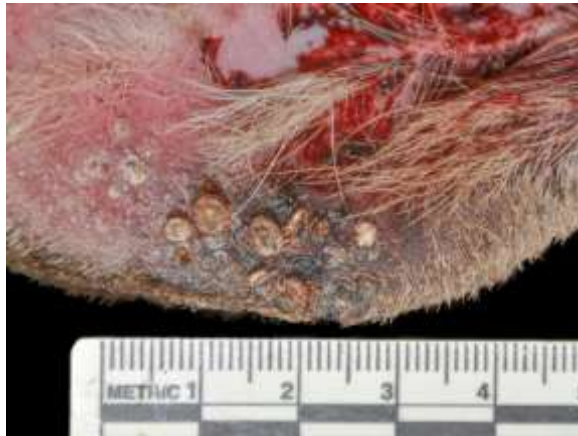
RK-268. *Mazama gouazoubira*. Female, adult. Tick, Family: *Ixodidae*, genus: *Amblyomma*.

RK-336. *Mazama gouazoubira*. Female, adult. Mesenterium. Cyst, Cestode.

Table 3. Microscopic findings of deer. Marsh deer (*Blastocerus dichotomus*, n=1), Brown brocket deer (*Mazama gouazoubira*, n=23).

Species, microscopic findings	N° cases
Blastocerus dichotomus (n=1)	
Esophagus	**1**
No significant findings	1
Heart	**1**
No significant findings	1
Kidney	**1**
Hemorrhage, cortex	1
Hemorrhage, medullae	1
Nephritis, interstitial, mononuclear (lymphocytic)	1
Liver	**1**
No significant findings	1
Lungs	**1**
Edema, alveolar	1
Pneumonia, granulomatous by lungworm	1
Rumen	**1**
No significant findings	1
Small intestine	**1**
Enteritis, mononuclear (Lymphocytic)	1
Spleen	**1**
Congestion	1
Testicle	**1**
Sexual maturity	1

Cont.

Mazama gouazoubira (n=23)

Abomasum		**11**
	No significant findings	9
	Autolysis	2
Adrenal glands		**10**
	Hemorrhage, pericapsular	1
	No significant findings	6
	Autolysis	3
Bone Marrow		**4**
	No significant findings	2
	Autolysis	2
Brain		**11**
	Hemorrhage, neuroparenchyma	2
	Encephalitis, nonsuppurative (lymphocytic)	1
	Perivascular cuffing, mononuclear (lymphocytic)	1
	No significant findings	3
	Autolysis	5
Carotid		**6**
	No significant findings	5
	Autolysis	1
Cerebellum		**3**
	No significant findings	2
	Autolysis	1
Diaphragm		**6**
	No significant findings	7
Epiglottis		**4**
	Protozoa, sarcocystid, Intrasarcolemal, no inflammation	1
	No significant findings	3
Esophagus		**13**
	No significant findings	13
Eye		**1**
	No significant findings	1
Heart		**22**
	Myocarditis, granulocytic (neutrophilic)	1
	Protozoa, sarcocystid, Intrasarcolemal, no inflammation	1
	No significant findings	17
	Autolysis	3
Jugular		**2**
	No significant findings	2
Kidney		**21**
	Leukocytic infiltrate, perivascular, mononuclear (lymphocytic)	1
	Necrosis, tubular, acute	1
	Nephritis, interstitial, mixed (neutrophilic, histiocytic)	1
	Proteinosis, glomerular/tubular	1
	Proteinosis, glomerular	1
	Proteinosis, tubular	1
	No significant findings	1
	Autolysis	18
Liver		**20**
	Hepatitis, lobular, mononuclear (lymphocytic)	5
	Pericholangitis, mononuclear (lymphocytic, histiocytic)	2
	Degeneration, steatosis, macrogoticular, hepatocyte	1
	Ductular reaction, bile duct	1
	Hemosiderosis	1
	Hepatitis, portal, mixed (lymphocytic, neutrophilic)	1
	Hepatitis, portal, mononuclear (lymphocytic, histiocytic)	1
	Leukocyte infiltration, periductular, mononuclear (lymphocytic)	1

Cont.

	Pericholangitis, granulocytic (eosinophilic)	1
	No significant findings	4
	Autolysis	9
Lungs		**23**
	Edema, alveolar	21
	Edema, perivascular	16
	Hemorrhage, alveolar	12
	Hemorrhage, perivascular	9
	Edema, interlobar	8
	Hemorrhage, interlobar	6
	Rupture, alveolar	6
	Bullae, alveolar	4
	Edema, subpleural	3
	Hyperinsuflation, alveolar	3
	Edema, interlobar	2
	BALT hyperplasia	1
	Bronchopneumonia, acute, granulocytic (neutrophilic)	1
	Bullae, subpleural	1
	Hemorrhage, subpleural	1
	Hemosiderosis	1
	Leukocyte infiltration, Interlobular interstitium, granulocytic (neutrophilic)	1
	Pneumonia, granulomatous	1
	Autolysis	1
Lymph node		**5**
	No significant findings	2
	Autolysis	4
Lymph node, mesenteric		**1**
	No significant findings	1
Mammary gland		**1**
	No significant findings	1
Nerve		**1**
	No significant findings	1
Omasum		**9**
	No significant findings	8
	Autolysis	1
Pancreas		**2**
	No significant findings	1
	Autolysis	1
Penis		**1**
	No significant findings	1
Reticulum		**13**
	No significant findings	12
	Autolysis	1
Rumen		**13**
	No significant findings	11
	Autolysis	2
Skeletal muscle		**18**
	Rupture, myofiber	4
	Degeneration, myofiber	2
	Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
	No significant findings	13
	Autolysis	3
Skeletal muscle, periocular		**1**
	Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
Skin		**8**
	Dermatitis, mixed (histiocytic, neutrophilic)	1
	No significant findings	7

Cont.

Small intestine		**7**
	Enteritis, granulocytic (eosinophylic)	1
	No significant findings	4
	Autolysis	3
Spine		**3**
	No significant findings	3
Spleen		**20**
	Hemosiderosis	7
	White pulp hyperplasia	4
	Hemorrhage, red pulp	3
	Histiocytosis, red pulp	1
	Necrosis, centrifollicular	1
	No significant findings	3
	Autolysis	5
Testicle		**6**
	Sexual maturity	6
Thymus		**1**
	No significant findings	1
Thyroid		**1**
	Autolysis	1
Tongue		**18**
	Glossitis, granulomatous by foreign body	2
	Glossitis, granulocytic (neutrophilic)	1
	Glossitis, internal, granulocytic (neutrophilic)	1
	Glossitis, internal, mixed (neutrophilic, histiocytic, lymphocytic)	1
	Glossitis, submucosal, granulocytic (neutrophilic)	1
	No significant findings	12
Tonsil		**1**
	Autolysis, advanced	1
Trachea		**14**
	No significant findings	14
Urinary bladder		**10**
	No significant findings	8
	Autolysis	2
Uterus		**3**
	No significant findings	3

APPENDIX II

General information, gross and histopathological findings in carnivores

<u>Canidae</u>

Table 1. General information of canids.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-024	*Cerdocyon thous*	Female	Adult	6	Regular	2017	June	Dry	MS	Tres Lagoas	Atlantic forest
RK-025	*Cerdocyon thous*	Male	Adult	6.3	Regular	2017	June	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-026	*Cerdocyon thous*	Female	Adult	-	Good	2017	July	Dry	MS	Dois Irmãos do Buriti	Cerrado
RK-027	*Cerdocyon thous*	Female	Adult	5.7	Good	2017	July	Dry	MS	Três Lagoas	Atlantic forest
RK-028	*Cerdocyon thous*	Female	Adult	-	Good	2017	July	Dry	MS	Bataguassu	Cerrado
RK-030	*Cerdocyon thous*	Male	Adult	-	Good	2017	July	Dry	MS	Campo Grande	Cerrado
RK-043	*Cerdocyon thous*	Female	Juvenile	5.12	Regular	2017	October	Rain	MS	Nova Andradina	Cerrado
RK-044	*Cerdocyon thous*	Female	Adult	5.83	Good	2017	October	Rain	MS	Bataguassu	Cerrado
RK-046	*Cerdocyon thous*	Male	Adult	6.7	Good	2017	October	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-063	*Cerdocyon thous*	Female	Infant	1.84	Regular	2017	November	Rain	MS	Nova Andradina	Cerrado
RK-068	*Cerdocyon thous*	Female	Adult	5.68	Good	2017	December	Rain	MS	Anastácio	Cerrado
RK-070	*Cerdocyon thous*	Female	Juvenile	3.1	Good	2017	December	Rain	MS	Miranda	Pantanal
RK-072	*Cerdocyon thous*	Female	Juvenile	3.14	Good	2017	December	Rain	MS	Anastácio	Cerrado
RK-077	*Cerdocyon thous*	Female	Juvenile	4.6	Good	2017	December	Rain	MS	Miranda	Pantanal
RK-079	*Cerdocyon thous*	Female	Adult	-	Good	2017	December	Rain	MS	Miranda	Pantanal
RK-087	*Cerdocyon thous*	Female	Adult	6.1	Good	2017	July	Dry	SP	Itapecerica da Serra	Atlantic forest
RK-212	*Cerdocyon thous*	Male	Adult	6.1	Good	2018	June	Dry	MS	Dois Irmãos do Buriti	Cerrado
RK-217	*Cerdocyon thous*	Female	Adult	-	Good	2018	May	Dry	MS	Anastácio	Cerrado
RK-220	*Cerdocyon thous*	Female	Adult	6.1	Good	2018	June	Dry	MS	Anastácio	Cerrado
RK-243	*Cerdocyon thous*	Male	Adult	6.67	Regular	2018	July	Dry	MS	Campo Grande	Cerrado
RK-247	*Cerdocyon thous*	Male	Adult	6.61	Good	2018	July	Dry	MS	Miranda	Pantanal
RK-254	*Cerdocyon thous*	Male	Adult	5.6	Good	2018	July	Dry	SP	Canitar	Cerrado
RK-255	*Cerdocyon thous*	Female	Adult	-	Good	2018	August	Dry	MS	Campo Grande	Cerrado
RK-258	*Cerdocyon thous*	Female	Adult	7.3	Good	2018	July	Dry	SP	Jambeiro	Atlantic forest
RK-265	*Cerdocyon thous*	Male	Adult	-	Good	2018	August	Dry	MS	Campo Grande	Cerrado
RK-274	*Cerdocyon thous*	Male	Adult	7.7	Good	2018	July	Dry	SP	São Lourenço da Serra	Atlantic forest
RK-292	*Cerdocyon thous*	Unknown	Infant	-	Good	2018	November	Rain	MS	Campo Grande	Cerrado
RK-297	*Cerdocyon thous*	Female	Adult	6	Good	2018	October	Rain	SP	Jambeiro	Atlantic forest
RK-298	*Cerdocyon thous*	Male	Adult	6.8	Good	2018	October	Rain	SP	Jambeiro	Atlantic forest
RK-299	*Cerdocyon thous*	Male	Adult	6.2	Good	2018	October	Rain	SP	Paraibuna	Atlantic forest
RK-309	*Cerdocyon thous*	Male	Juvenile	4.7	Good	2019	February	Rain	SP	Paraibuna	Atlantic forest
RK-318	*Cerdocyon thous*	Male	Adult	-	Good	2018	September	Dry	MS	Campo Grande	Cerrado
RK-335	*Cerdocyon thous*	Female	Juvenile	5.32	Good	2018	March	Rain	PR	Rio Negro	Atlantic forest
RK-339	*Cerdocyon thous*	Male	Adult	6	Good	2017	June	Dry	SC	Unknown	Atlantic forest
RK-349	*Cerdocyon thous*	Female	Adult	6.3	Good	2015	March	Rain	SC	São Francisco do Sul	Atlantic forest
RK-351	*Cerdocyon thous*	Female	Adult	6.34	Good	2015	August	Dry	SC	São Francisco do Sul	Atlantic forest
RK-356	*Cerdocyon thous*	Female	Adult	5.46	Good	2017	May	Dry	SC	São Francisco do Sul	Atlantic forest
RK-362	*Cerdocyon thous*	Female	Adult	5.5	Good	2012	October	Rain	SC	São Francisco do Sul	Atlantic forest

RK-366	*Cerdocyon thous*	Female	Juvenile	2.3	Good	2016	November	Rain	SC	Papanduva	Atlantic forest
RK-367	*Cerdocyon thous*	Male	Adult	7.27	Good	2007	July	Dry	SC	Araranguá	Atlantic forest
RK-371	*Cerdocyon thous*	Male	Juvenile	2.69	Good	2017	January	Rain	SC	Papanduva	Atlantic forest
RK-376	*Cerdocyon thous*	Female	Juvenile	4.39	Good	2017	February	Rain	PR	Rio Negro	Atlantic forest
RK-390	*Cerdocyon thous*	Male	Juvenile	4.98	Good	2011	June	Dry	SC	São Francisco do Sul	Atlantic forest
RK-391	*Cerdocyon thous*	Female	Adult	6.55	Good	2015	May	Dry	SC	São Francisco do Sul	Atlantic forest
RK-393	*Cerdocyon thous*	Male	Adult	7.82	Good	2019	April	Dry	PR	Rio Negro	Atlantic forest
RK-396	*Cerdocyon thous*	Male	Adult	6.57	Good	2018	July	Dry	PR	Campo do Tenente	Atlantic forest
RK-412	*Cerdocyon thous*	Female	Adult	6	Good	2019	June	Dry	SP	Paraibuna	Atlantic forest
RK-423	*Cerdocyon thous*	Male	Adult	6.5	Good	2019	June	Dry	SP	Paraibuna	Atlantic forest
RK-449	*Cerdocyon thous*	Unknown	Adult	-	Good	2019	December	Rain	MS	Miranda	Pantanal
RK-083	*Chrysocyon brachyurus*	Female	Adult	22.5	Regular	2018	January	Rain	SP	Unknown	Atlantic forest
RK-090	*Chrysocyon brachyurus*	Female	Adult	24.5	Good	2018	January	Rain	SP	Unknown	Atlantic forest
RK-250	*Chrysocyon brachyurus*	Male	Juvenile	20	Good	2018	May	Dry	SP	Paraibuna	Atlantic forest
RK-272	*Chrysocyon brachyurus*	Male	Adult	26.4	Good	2018	November	Rain	SP	Unknown	Atlantic forest
RK-320	*Chrysocyon brachyurus*	Male	Adult	22.5	Good	2019	March	Rain	MS	Campo Grande	Cerrado
RK-029	*Lycalopex vetulus*	Male	Adult	2.9	Good	2017	July	Dry	MS	Nova Andradina	Cerrado
RK-211	*Lycalopex vetulus*	Male	Adult	3.8	Good	2018	June	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-437	*Lycalopex vetulus*	Male	Juvenile	-	Good	2019	September	Dry	MS	Campo Grande	Cerrado
RK-408	*Speothos venaticus*	Male	Adult	6.35	Good	2019	August	Dry	MS	Aquidauana	Pantanal

Figure 1. Spatial distribution, and main characteristics of crab-eating foxes.
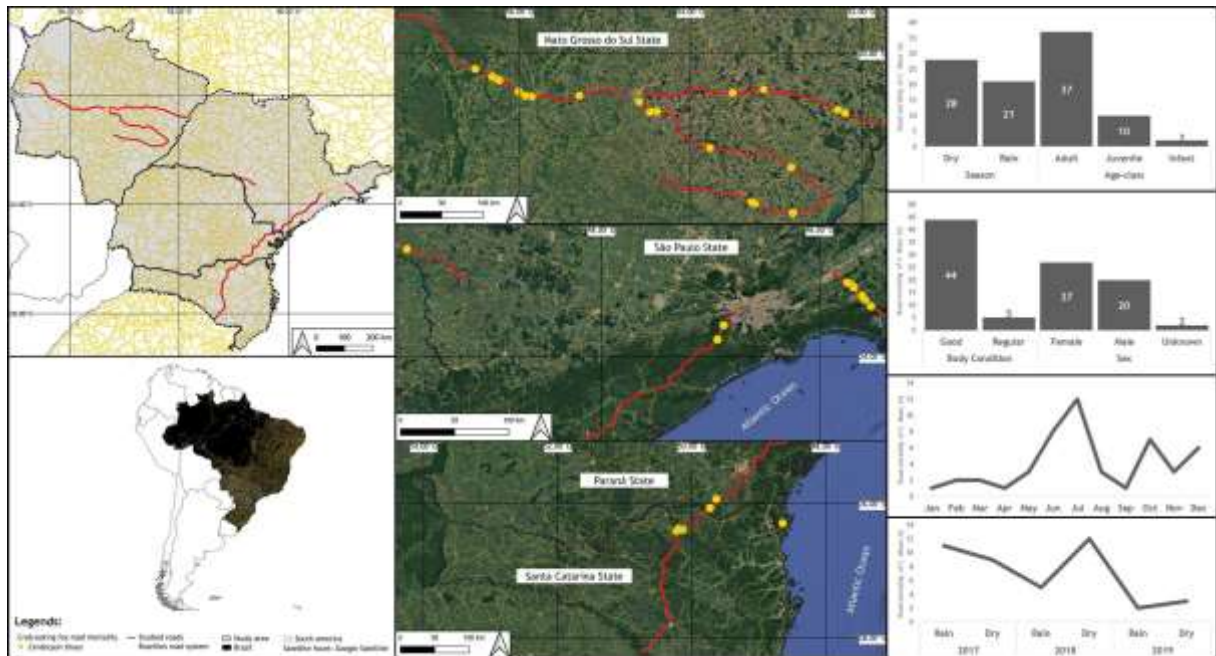
Figure 2. Spatial distribution, and main characteristics of other canids.
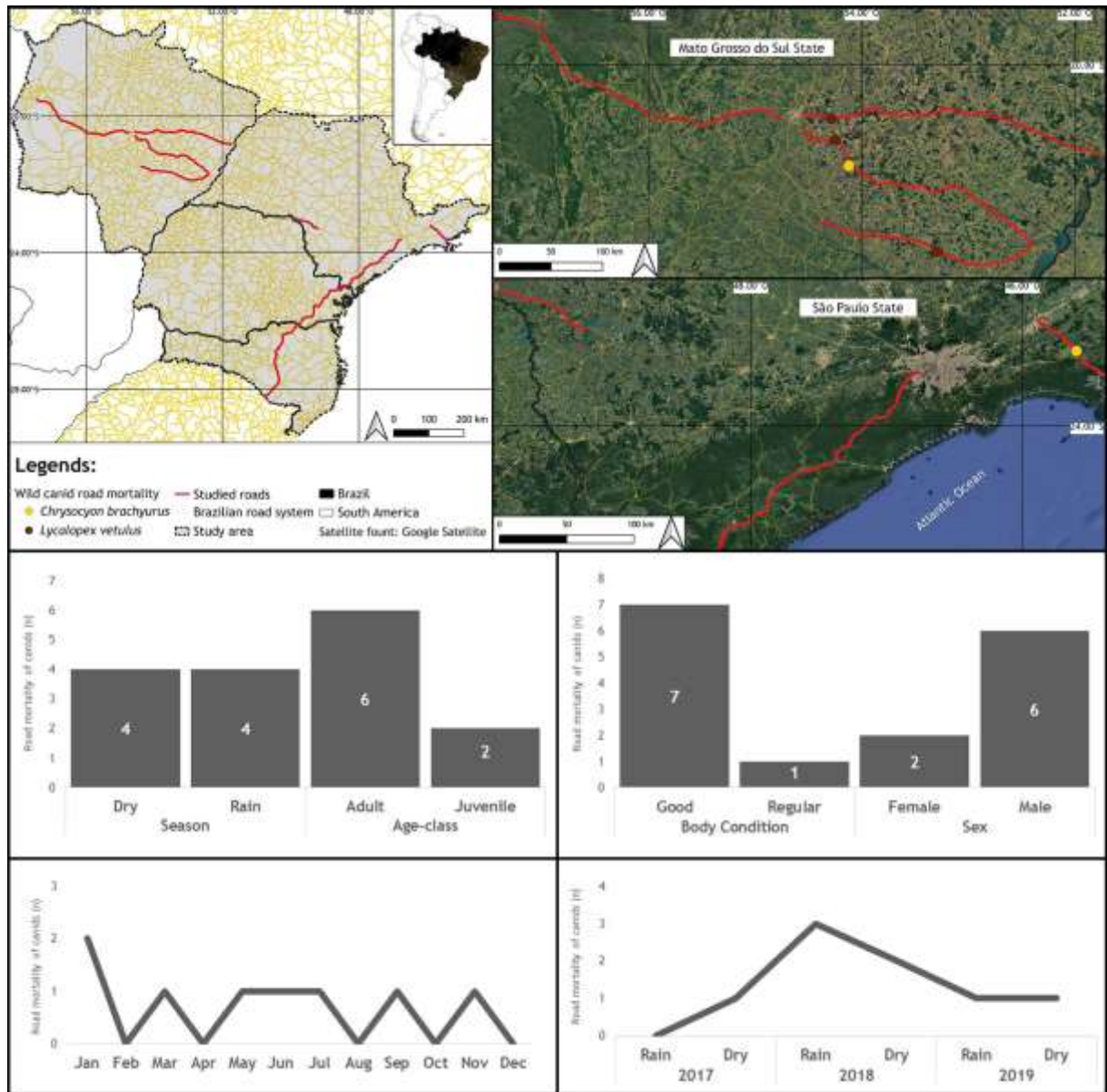
Table 2.   Gross findings non-associated with trauma in canids.

Species, gross finding	Cases
***Cerdocyon thous* (n=44)**	
Lymph node megaly	27% (12/44)
White pulp hyperplasia, spleen	18% (8/44)
Splenomegaly	11% (5/44)
Tick infestation	9% (4/44)
Predation	5% (2/44)
Endoparasites in Esophagus	2% (1/44)
Flea infestation	2% (1/44)
Small intestine, endoparasitic infestation	2% (1/44)
***Chrysocyon brachyurus* (n=5)**	
Tick infestation	40% (2/5)
Unilateral hydronephrosis (right) by Dyoctophyma sp.	40% (2/5)
Splenomegaly	20% (1/5)
Gallbladder, distended	20% (1/5)
Lymph node megaly	20% (1/5)
***Lycalopex vetulus* (n=1)**	
Tick infestation	100% (1/1)
***Spheotos venaticus* (n=1)**	
Lymph node megaly	100% (1/1)
Parasitic gastritis by nematodes	100% (1/1)
Pulmonary parasitic granuloma	100% (1/1)

Figure 3. Main gross findings in canids.
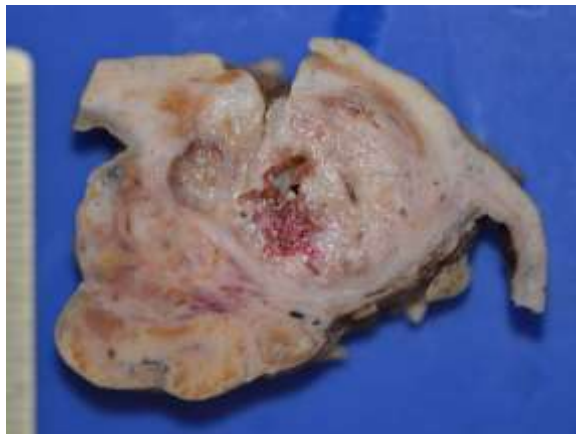
RK-408. *Speothos venaticus*. Male, adult. Stomach. Nodular parasitic gastritis. Nematode

RK-408. *Speothos venaticus.* Male, adult. Lungs. Nodular metazoan cyst. Cestode.

Table 3. Microscopic findings of canids. Crab-eating fox (*Cerdocyon thous*, n=47), Maned wolf (*Chrysocyon brachyurus*, n=5), Hoary fox (*Lycalopex vetulus*, n=2), Bush dog (*Spheotos venaticus*, n=1).

Species, microscopic findings	Cases
***Cerdocyon thous* (n=47)**	
Adipose tissue	**1**
Steatitis, mixed (neutrophilic, histiocytic)	1
Adrenal glands	**18**
Vacuolar degeneration, cortex	2
Hemorrhage, cortical	1
Hemorrhage, pericapsular	1
Loss and fibrosis, cortex, fascicular/reticular	1
No significant findings	6
Autolysis	9
Brain	**14**
Neuron, hipereosinofilia	2
Hemorrhage, leptomeningeal	3
Gliosis	1
Hemorrhage, neuroparenchyma	1
Perivascular cuffing, mononuclear (lymphocytic)	1
No significant findings	7
Autolysis	2
Carotid	**7**
Hemorrhage, pericapsular	1
No significant findings	6
Cecum	**1**
Autolysis	1
Cerebellum	**5**
No significant findings	5
Colon	**1**
No significant findings	1
Diaphragm	**11**
Rupture, myofiber	6
Degeneration, myofiber	3
Hemorrhage, interstitial	1
Hypercontraction, myofiber	1
Protozoa, sarcocystid, intrasarcolemal, no inflammation	1
No significant findings	5
Esophagus	**29**
Esophagitis, granulocytic (neutrophilic)	2
Hemorrhage, periserosal	1
Leukocytic infiltrate, perivascular, mononuclear (lymphocytic, histiocytic)	1
Protozoa, sarcocystid, intrasarcolemal, no inflammation	1
No significant findings	22
Autolysis	2
Eye	**2**
No significant findings	2
Heart	**39**
Degeneration, cardiomyocyte	1
Hemorrhage, myocardial	2
Myocarditis, mononuclear (lymphocytic, histiocytic)	2
No significant findings	23
Autolysis	11
Jugular	**4**
No significant findings	4

	Cont.
Kidney	**39**
Hemorrhage, cortex	5
Nephritis, interstitial, mononuclear (lymphocytic, histiocytic, plasma cells)	3
Pyelonephritis, interstitial, mononuclear (lymphocytic, histiocytic)	2
Congestion, medullar	1
Degeneration, tubular	1
Hemorrhage, pericapsular	1
Pyelitis, suppurative (neutrophilic)	1
No significant findings	5
Autolysis	24
Large intestine	**12**
Colitis, lamina propria, mononuclear (lymphocytic)	2
No significant findings	5
Autolysis	6
Liver	**34**
Degeneration, hydropic, hepatocyte	5
Metazoan, trematode, larvae, adult, intraductal	5
Cyst, lobular, cestode, hydatid	3
Hepatitis, lobular, mononuclear (lymphocytic, histiocytic)	3
Congestion, sinusoidal	2
Hemosiderosis	2
Hepatitis, portal, mixed (eosinophilic, lymphocytic, histiocytic)	2
Pericholangitis, mononuclear (lymphocytic, histiocytic)	2
Degeneration, steatosis, macrogoticular, hepatocyte	1
Hemorrhage, lobular	1
Hemorrhage, portal	1
Leukocyte infiltration, sinusoids, mixed (neutrophilic, lymphocytic)	1
Necrosis/apoptosis, hepatocyte, single cell	1
Pericholangitis, granulocytic (eosinophilic, neutrophilic)	1
No significant findings	6
Autolysis	21
Lungs	**44**
Edema, alveolar	35
Hemorrhage, alveolar	33
Edema, perivascular	31
Hemorrhage, perivascular	31
Congestion, capillary beds	12
Artery, tunica media, hypertrophy	11
Hemosiderosis	7
Pneumonia, granulomatous by lungworm	7
Anthracosis	4
Bullae, subpleural	4
Histiocytosis, alveolar	4
Pneumonia, bronchointerstitial, mononuclear (lymphocytic, histiocytic) with MNGC	4
Pneumonia, interstitial, mononuclear (lymphocytic, histiocytic)	4
Bullae, alveolar	3
Bronchopneumonia, acute, granulocytic (neutrophilic)	2
Edema, subpleural	2
Hemorrhage, bronchus	2
Hemorrhage, subpleural	2
Metazoan, nematode, larvae, adult, alveolar septae, vascular lumen	2
Metazoan, nematode, larvae, adult/L1 and eggs, alveolar lumen/septae	2
Leukocyte infiltration, perivascular, mononuclear (lymphocytic, histiocytic)	1
Metazoan, nematode, larvae, adult, alveolar lumen, no inflammation	1

		Cont.
	Pleuritis, granulomatous by lungworm	1
	Pleuritis, mononuclear (lymphocytic, histiocytic), chronic	1
	Pneumonia, pyogranulomatous by adiaspores (Emmonsia)	1
	Rupture, alveolar	1
	Autolysis	4
Lymph node		**15**
	Paracortical lymphoid reactive hyperplasia	4
	Hemorrhage, sinusal	1
	No significant findings	5
	Autolysis	7
Lymph node, mesenteric		**12**
	Hemosiderosis	3
	Paracortical lymphoid reactive hyperplasia	2
	Histiocytosis, sinusal	1
	Metazoan, nematode, microfilaries, intravascular, no inflammation	1
	Autolysis	7
Lymph node, tracheal		**1**
	Hemosiderosis	1
Ovary		**2**
	No significant findings	2
Pancreas		**13**
	No significant findings	9
	Autolysis	4
Prostate		**1**
	No significant findings	1
Salivary gland		**5**
	Adenitis, mononuclear (lymphocytic)	1
	No significant findings	4
Skeletal muscle		**18**
	Degeneration, myofiber	6
	Rupture, myofiber	6
		Cont.
	Necrosis, myofiber	1
	Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
	No significant findings	11
Skin		**4**
	No significant findings	3
	Autolysis	1
Small intestine		**31**
	MALT hyperplasia	7
	Enteritis, granulocytic (eosinophilic)	2
	Enteritis, mononuclear (Lymphocytic)	2
	Metazoan, cestode, larvae, adult, lumen, no inflammation	1
	No significant findings	12
	Autolysis	14
Spine		**2**
	No significant findings	2
Spleen		**34**
	Hemorrhage, red pulp	7
	White pulp hyperplasia	7
	Hemosiderosis	4
	White pulp depletion	2
	Histiocytosis, red pulp	1
	Splenitis, granulocytic (neutrophilic)	1
	No significant findings	5
	Autolysis	13

	Cont.
Stomach	**16**
Gastritis, granulocytic (eosinophilic)	3
Gastritis, granulomatous	1
Hemorrhage, submucosa	1
No significant findings	7
Autolysis	5
Testicle	**8**
Sexual maturity	5
Azoospermia	1
Hemorrhage	1
Orquitis, mononuclear (lymphocytic)	1
No significant findings	1
Thymus	**4**
No significant findings	3
Autolysis	2
Thyroid	**2**
No significant findings	1
Autolysis	1
Tongue	**19**
Glossitis, internal, mixed (neutrophilic, histiocytic, lymphocytic)	2
Degeneration, myofiber	1
Glossitis, granulomatous by foreign body	1
Glossitis, mononuclear (lymphocytic)	1
Glossitis, superficial, mixed (eosinophilic, lymphocytic)	1
Hemorrhage, muscular layer	1
Metazoan, nematode, Capillaria sp., larvae, adult and eggs, epithelium	1
No significant findings	13
Tonsil	**1**
No significant findings	1
Trachea	**34**
Tracheitis, mononuclear (lymphocytic, histiocytic)	2
Hemorrhage, serosa	1
No significant findings	29
Autolysis	2
Urinary bladder	**15**
Hemorrhage	1
No significant findings	11
Autolysis	3
Uterus	**3**
No significant findings	3
***Chrysocyon brachyurus* (n=5)**	
Diaphragm	**4**
Protozoa, sarcocystid, intrasarcolemal, no inflammation	1
No significant findings	2
Autolysis	1
Duodenum	**1**
Autolysis	1
Esophagus	**2**
No significant findings	1
Autolysis	1
Heart	**2**
No significant findings	2
Kidney	**4**
Fibrosis	1
Nephritis, interstitial, mixed (lymphocytic, histiocytic, eosinophilic)	1
Nephritis, interstitial, mononuclear (lymphocytic)	1
Autolysis	3

Cont.

Large intestine	**2**
Autolysis	2
Liver	**4**
Autolysis	4
Lungs	**5**
Edema, alveolar	2
Hemorrhage, alveolar	2
BALT hyperplasia	1
Congestion, capillary beds	1
Edema, perivascular	1
Edema, subpleural	1
Hemorrhage, perivascular	1
Hemosiderosis	1
Pneumonia, interstitial, granulocytic (neutrophilic), acute	1
Autolysis	4
Pancreas	**1**
Autolysis	1
Salivary gland	**1**
No significant findings	1
Skeletal muscle	**3**
No significant findings	1
Autolysis	2
Small intestine	**4**
Autolysis	4
Spleen	**3**
Hemorrhage, red pulp	1
Hemosiderosis	1
Autolysis	2
Stomach	**2**
No significant findings	1
Autolysis	1
Testicle	**2**
Azoospermia	1
No significant findings	1
Tongue	**2**
No significant findings	2
Trachea	**4**
No significant findings	2
Autolysis	2
Urinary bladder	**1**
No significant findings	1
Lycalopex vetulus **(n=2)**	
Adrenal glands	**2**
Hemorrhage, cortical, glomerular	1
Hemorrhage, pericapsular	1
Vacuolar degeneration, cortex, fascicular	1
Brain	**2**
Congestion	1
Hemorrhage, leptomeningeal	1
Cerebellum	**2**
Hemorrhage, leptomeningeal	1
No significant findings	1
Diaphragm	**1**
No significant findings	1
Esophagus	**2**
No significant findings	2

		Cont.
Heart		**2**
	No significant findings	2
Kidney		**2**
	Hemorrhage, corticomedular	1
	No significant findings	1
	Autolysis	1
Large intestine		**1**
	No significant findings	1
Liver		**2**
	Hepatitis, lobular, mononuclear (lymphocytic, histiocytic)	1
	Hepatitis, portal, mononuclear (lymphocytic, histiocytic)	1
	Metazoan, trematode, larvae, adult, intraductal, no inflammation	1
	Autolysis	1
Lungs		**2**
	Edema, alveolar	2
	Edema, perivascular	2
	Hemorrhage, alveolar	2
	Hemorrhage, perivascular	2
	Artery, tunica media, hypertrophy	1
	Congestion, capillary beds	1
	Hyperinsuflation, alveolar	1
	Rupture, alveolar	1
	Autolysis	1
Skeletal muscle		**1**
	Rupture, myofiber	1
Skin		**1**
	No significant findings	1
Small intestine		**2**
	Hemorrhage, serosa	1
	No significant findings	1
	Autolysis	1
Spleen		**2**
	Hemorrhage, red pulp	1
	Autolysis	1
Stomach		**2**
	Gastritis, granulocytic (eosinophilic)	1
	No significant findings	1
Tongue		**1**
	No significant findings	1
Trachea		**1**
	No significant findings	1
Urinary bladder		**1**
	No significant findings	1

Spheotos venaticus (n=1)		
Carotid		**1**
	No significant findings	1
Duodenum		**1**
	MALT hyperplasia	1
Esophagus		**1**
	No significant findings	1
Heart		**1**
	Myocarditis, pyogranulomatous (histiocytic, neutrophilic) by protozoa	1
Kidney		**1**
	Nephritis, interstitial, mononuclear (lymphocytic)	1
	Proteinosis, glomerular	1
	Proteinosis, tubular	1
	Proteinosis, tubular/glomerular	1

	Cont.
Large intestine	**1**
Colitis, lamina propria, mixed (eosinophilic, histiocytic, lymphocytic) by nematodes	1
Liver	**1**
Fibrosis, portal	1
Hemosiderosis	1
Autolysis	1
Lungs	**1**
Anthracosis	1
Edema, perivascular	1
Hemorrhage, alveolar	1
Hemorrhage, perivascular	1
Hemosiderosis	1
Parasitic cyst by cestode	1
Pneumonia, granulomatous by lungworm	1
Lymph node	**1**
Hemosiderosis	1
Plasmocitosis	1
Pancreas	**1**
No significant findings	1
Skeletal muscle	**1**
No significant findings	1
Skin	**1**
Fasciitis, mixed (neutrophilic, histiocytic, lymphocytic) by cestode	1
Folliculitis, mixed (neutrophilic, histiocytic)	1
Small intestine	**1**
Enteritis, lamina propria, granulocytic (eosinophilic) by nematodes	1
Metazoan, cestode, larvae, adult, lumen, no inflammation	1
Spleen	**1**
Splenitis, granulomatous by cestode eggs	1
Stomach	**1**
Gastritis, proliferative and fibrosing, eosinophilic with intralesional nematodes	1
Trachea	**1**
Hemorrhage, serosa	1
Urinary bladder	**1**
No significant findings	1

Felidae

Table 4. General information of felids.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-039	*Leopardus guttulus*	Female	Juvenile	1.89	Good	2017	February	Rain	SP	Caraguatatuba	Atlantic forest
RK-303	*Leopardus guttulus*	Male	Juvenile	2.2	Good	2018	October	Rain	SP	Paraibuna	Atlantic forest
RK-338	*Leopardus guttulus*	Female	Juvenile	2	Good	2018	January	Rain	PR	Quitandinha	Atlantic forest
RK-352	*Leopardus guttulus*	Male	Adult	3.76	Good	2016	February	Rain	SC	Itapoá	Atlantic forest
RK-354	*Leopardus guttulus*	Male	Juvenile	2.29	Good	2015	September	Dry	PR	São José dos Pinhais	Atlantic forest
RK-360	*Leopardus guttulus*	Male	Adult	2.71	Good	2016	July	Dry	SC	São Francisco do Sul	Atlantic forest
RK-369	*Leopardus guttulus*	Male	Juvenile	2.42	Good	2019	January	Rain	SC	Itaiópolis	Atlantic forest
RK-032	*Leopardus pardalis*	Male	Juvenile	10.9	Good	2017	April	Dry	SP	Paraibuna	Atlantic forest

| RK-066 | *Leopardus pardalis* | Male | Adult | 13.6 | Good | 2017 | November | Rain | SP | Caraguatatuba | Atlantic forest |
RK-067	*Leopardus pardalis*	Male	Juvenile	6.1	Good	2017	April	Dry	SP	Miracatu	Atlantic forest
RK-175	*Leopardus pardalis*	Female	Adult	9.8	Good	2018	January	Rain	SP	Caraguatatuba	Atlantic forest
RK-216	*Leopardus pardalis*	Male	Adult	-	Good	2018	May	Dry	MS	Santa Rita do Pardo	Cerrado
RK-263	*Leopardus pardalis*	Male	Juvenile	3	Good	2018	September	Dry	SP	Ipaussu	Cerrado
RK-273	*Leopardus pardalis*	Female	Juvenile	4.8	Regular	2018	October	Rain	SP	Barra do Turvo	Atlantic forest
RK-276	*Leopardus pardalis*	Male	Adult	8.7	Good	2018	June	Dry	SP	Cajati	Atlantic forest
RK-278	*Leopardus pardalis*	Male	Adult	13.5	Good	2018	October	Rain	SP	Barra do Turvo	Atlantic forest
RK-310	*Leopardus pardalis*	Male	Adult	13.4	Good	2019	February	Rain	SP	Caraguatatuba	Atlantic forest
RK-332	*Leopardus pardalis*	Male	Adult	15.8	Good	2019	May	Dry	SC	Capão Alto	Atlantic forest
RK-400	*Leopardus pardalis*	Male	Adult	10.3	Good	2019	June	Dry	SP	Paraibuna	Atlantic forest
RK-407	*Leopardus pardalis*	Male	Adult	-	Good	2019	August	Dry	MS	Anastácio	Cerrado
RK-414	*Leopardus pardalis*	Male	Adult	13	Good	2019	June	Dry	SP	Paraibuna	Atlantic forest
RK-344	*Leopardus wiedii*	Male	Adult	3.66	Good	2014	April	Dry	SC	São Francisco do Sul	Atlantic forest
RK-397	*Leopardus wiedii*	Male	Adult	3.19	Good	2014	March	Rain	SC	Itapema	Atlantic forest
RK-034	*Puma concolor*	Male	Adult	50	Good	2017	September	Dry	SP	Caraguatatuba	Atlantic forest
RK-042	*Puma concolor*	Female	Juvenile	22	Regular	2017	September	Dry	SP	Piracicaba	Atlantic forest
RK-206	*Puma concolor*	Female	Juvenile	27.7	Good	2018	January	Rain	SP	Registro	Atlantic forest
RK-280	*Puma concolor*	Male	Juvenile	30.3	Good	2018	November	Rain	SP	Cajati	Atlantic forest
RK-062	*Puma yagouaroundi*	Male	Juvenile	3.2	Good	2017	November	Rain	SP	Jambeiro	Atlantic forest
RK-088	*Puma yagouaroundi*	Male	Adult	3.9	Good	2018	January	Rain	SP	Unknown	Atlantic forest
RK-089	*Puma yagouaroundi*	Male	Juvenile	3.1	Regular	2018	January	Rain	SP	Unknown	Atlantic forest
RK-205	*Puma yagouaroundi*	Male	Adult	6.7	Good	2018	January	Rain	SP	Paraibuna	Atlantic forest
RK-215	*Puma yagouaroundi*	Female	Adult	5	Good	2018	April	Dry	MS	Anastácio	Cerrado
RK-218	*Puma yagouaroundi*	Male	Adult	7.07	Good	2018	February	Rain	MS	Campo Grande	Cerrado
RK-300	*Puma yagouaroundi*	Male	Adult	6	Good	2019	February	Rain	SP	Bernardino de Campos	Cerrado
RK-323	*Puma yagouaroundi*	Male	Adult	-	Good	2019	February	Rain	MS	Aquidauana	Pantanal
RK-359	*Puma yagouaroundi*	Female	Adult	3.74	Good	2017	September	Dry	SC	Itaiópolis	Atlantic forest
RK-375	*Puma yagouaroundi*	Female	Adult	4.61	Good	2016	November	Rain	SC	Monte Castelo	Atlantic forest
RK-398	*Puma yagouaroundi*	Male	Adult	6.14	Good	2011	October	Rain	SC	São Francisco do Sul	Atlantic forest
RK-404	*Puma yagouaroundi*	Male	Adult	6.4	Good	2019	August	Dry	MS	Terenos	Cerrado

Figure 4. Spatial distribution, and main characteristics of felids.
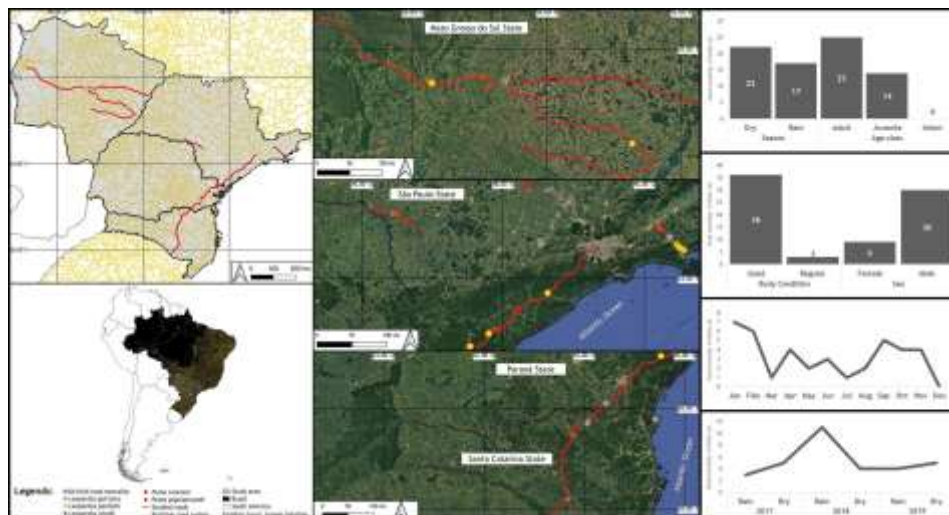
Table 5. Gross findings non-associated with trauma in felids.

Species, gross finding	Cases
Leopardus guttulus (n=7)	
Small intestine, endoparasitic infestation	43% (3/7)
Mesenteric lymph node megaly	43% (3/7)
Consolidated fracture	14% (1/7)
Splenomegaly	14% (1/7)
Flea infestation	14% (1/7)
Lungs, metazoan parasites intrabronchial	14% (1/7)
Passive hepatic congestion	14% (1/7)
Leopardus pardalis (n=13)	
Mesenteric lymph node megaly	38% (5/13)
Splenomegaly	23% (3/13)
Small intestine, endoparasitic infestation	23% (3/13)
Bilateral hydronephrosis	8% (1/13)
Hepatic necrosis	8% (1/13)
Mucous tracheitis	8% (1/13)
Parasitic enteritis	8% (1/13)
Tick infestation	8% (1/13)
Leopardus tigrinus (n=2)	
Mesenteric lymph node megaly	50% (1/2)
Passive hepatic congestion	50% (1/2)
Leopardus wiedii (n=2)	
Consolidated fracture	50% (1/2)
Splenomegaly	50% (1/2)
Mesenteric lymph node megaly	50% (1/2)
Puma concolor (n=4)	
Enteritis by nematodes	100% (4/4)
Enteritis by acanthocephala	100% (4/4)
Small intestine, endoparasitic infestation	50% (2/2)
Subcutaneous nematode migration	50% (2/2)
Tick infestation	50% (2/2)
Splenomegaly	25% (1/1)
Mesenteric lymph node megaly	25% (1/1)
Puma yagouaroundi (n=12)	
Mesenteric lymph node megaly	25% (3/12)
Enteritis by acanthocephala	8% (1/12)
Splenomegaly	8% (1/12)
Enteritis by nematodes	8% (1/12)
Pregnancy	8% (1/12)
Small intestine, endoparasitic infestation	8% (1/12)
Tick infestation	8% (1/12)

Figure 5. Main gross findings in felids.

RK280. *Puma concolor*. Male, adult. Subcutaneous. Adult metazoan worm. Nematoda

RK280. *Puma concolor*. Male, adult. Subcutaneous. Adult metazoan worm. Nematoda

RK-034. *Puma concolor*. Male, adult. Stomach. nodular parasitic gastritis. Nematoda

RK-215. *Puma jagouaroundi*. Female, adult. Stomach. nodular parasitic enteritis. Nematoda.

RK-034. *Puma concolor*. Male, adult. Stomach. nodular parasitic enteritis. Acanthocephala.

Table 6. Microscopic findings of felids. Southern tiger cat (*Leopardus guttulus*, n=8), Ocelot (*Leopardus pardalis*, n=12), Margay (*Leopardus wiedii*, n=2), Mountain lion (*Puma concolor*, n=4), Jaguarundi (*yagouaroundi*, n=12).

Species, microscopic findings	Cases
Leopardus guttulus (n=8)	
Adrenal glands	**1**
No significant findings	1
Carotid	**5**
No significant findings	4
Autolysis	1
Cerebellum	**1**
No significant findings	1
Diaphragm	**1**
No significant findings	1
Epiglottis	**1**
No significant findings	1
Esophagus	**7**
No significant findings	6
Autolysis	1
Heart	**8**
No significant findings	5
Autolysis	3
Jugular	**5**
No significant findings	4
Autolysis	1
Kidney	**7**
No significant findings	1
Autolysis	6
Large intestine	**2**
No significant findings	1
Autolysis	1
Liver	**6**
No significant findings	3
Autolysis	3
Lungs	**8**
Edema, alveolar	8
Metazoan, nematode, larvae, adult/L1 and eggs, alveolar lumen/septae	7
Artery, tunica media, hypertrophy	6
Bullae, subpleural	3
Hemorrhage, alveolar	3
Hemorrhage, perivascular	3
Bullae, alveolar	2
Edema, perivascular	2
Edema, subpleural	1
Emboli, nervous tissue	1
Autolysis	2
Lymph node	**4**
Autolysis,	4
Parathyroid	**1**
No significant findings	1
Skeletal muscle	**5**
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
No significant findings	3
Autolysis	1
Skin	**1**
No significant findings	1

Cont.

Small intestine **6**

Acanthocephala metazoans, intraluminal 1

Leiomyositis, mixed (eosinophilic, histiocytic) by acanthocephala 1

No significant findings 1

Autolysis 3

Spleen **6**

No significant findings 1

Autolysis 5

Stomach **3**

No significant findings 1

Autolysis 2

Testicle **4**

Sexual maturity 4

Thyroid **2**

No significant findings 2

Tongue **5**

Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation 2

No significant findings 1

Autolysis 2

Trachea **7**

No significant findings 6

Autolysis 1

Urinary bladder **4**

No significant findings 3

Autolysis 1

Leopardus pardalis **(n=12)**

Adipose tissue **1**

No significant findings 1

Adrenal glands **4**

Hemorrhage, pericapsular 1

Steatosis, cortex, fascicular 1

No significant findings 1

Autolysis 1

Brain **2**

No significant findings 1

Autolysis 1

Diaphragm **5**

Protozoa, sarcocystid, intrasarcolemal, no inflammation 1

No significant findings 4

Autolysis 1

Esophagus **5**

Leukocytic infiltrate, perivascular, mononuclear (lymphocytic) 1

No significant findings 4

Eye **1**

No significant findings 1

Heart **9**

Myocarditis, mononuclear (lymphocytic) 2

Myocarditis, pyogranulomatous (histiocytic, neutrophilic) by protozoa 1

No significant findings 4

Autolysis 3

Kidney **10**

Mineralization, tubular, medullae 1

No significant findings 2

Autolysis 7

Large intestine **5**

Colitis, mixed (eosinophilic, lymphocytic) 1

MALT hyperplasia 1

		Cont.
	Autolysis	4
Larynx		**1**
	No significant findings	1
Liver		**10**
	Degeneration, steatosis, microgoticular, hepatocyte	3
	Detrabeculation, hepatocytic cord	1
	Hemorrhage, lobular	1
	Hepatitis, lobular/portal, mononuclear (lymphocytic, histiocytic)	1
	Leukocyte infiltration, portal, mononuclear (lymphocytic)	1
	No significant findings	2
	Autolysis	5
Lungs		**10**
	Edema, alveolar	9
	Artery, tunica media, hypertrophy	8
	Edema, perivascular	5
	Hemorrhage, alveolar	5
	Edema, subpleural	3
	Hemorrhage, perivascular	3
	Metazoan, nematode, larvae, adult/L1, alveolar lumen/septae	3
	Congestion, capillary beds	2
	Hemosiderosis	2
	Hyperinsuflation, alveolar	2
	Bullae, alveolar	1
	Bullae, subpleural	1
	Leukocyte infiltration, perivascular, mononuclear (lymphocytic)	1
	Pneumonia, granulomatous by lungworm	1
	Pneumonia, interstitial, mixed (eosinophilic, lymphocytic)	1
	Pneumonia, subpleural, mixed (neutrophilic, lymphocytic, histiocytic)	1
	Autolysis	1
Lymph node		**7**
	Paracortical lymphoid reactive hyperplasia	2
	No significant findings	2
	Autolysis	3
Lymph node, mesenteric		**2**
	Lymphadenitis, granulomatous	1
	Paracortical lymphoid reactive hyperplasia	1
	Autolysis	1
Pancreas		**2**
	No significant findings	2
Parathyroid		**1**
	No significant findings	1
Prostate		**1**
	Autolysis	1
Salivary gland		**1**
	Autolysis	1
Skeletal muscle		**7**
	Degeneration, myofiber	1
	Rupture, myofiber	1
	No significant findings	4
	Autolysis	2
Skin		**2**
	No significant findings	2
Small intestine		**8**
	MALT hyperplasia	2
	Enteritis, granulocytic (eosinophilic)	1
	Enteritis, lamina propria, mononuclear (lymphocytic)	1
	Leiomyositis, granulocytic (eosinophilic)	1

		Cont.
	Leiomyositis, necrotic, mixed (eosinophilic, histiocytic)	1
	Metazoan, nematode, larvae, adult, lumen, no inflammation	1
	Serositis, granulocytic (eosinophilic)	1
	No significant findings	2
	Autolysis	5
Spleen		**10**
	Hemorrhage, red pulp	1
	No significant findings	5
	Autolysis, advanced	3
	Autolysis, moderate	2
Stomach		**8**
	No significant findings	5
	Autolysis, advanced	3
Testicle		**8**
	Sexual maturity	6
	Azoospermia	1
	Degeneration, tubules	1
	Leydig cell hyperplasia	1
	No significant findings	1
Thyroid		**2**
	No significant findings	2
Tongue		**6**
	Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
	No significant findings	5
Trachea		**7**
	No significant findings	6
	Autolysis	1
Urinary bladder		**5**
	Hemorrhage, mucosa	1
	No significant findings	2
	Autolysis	2

***Leopardus wiedii* (n=2)**

Carotid		**1**
	No significant findings	1
Esophagus		**2**
	No significant findings	1
	Autolysis	1
Heart		**2**
	No significant findings	2
Jugular		**1**
	No significant findings	1
Kidney		**2**
	Autolysis	2
Liver		**2**
	Autolysis	2
Lungs		**2**
	Edema, alveolar	1
	Edema, perivascular	1
	Hemorrhage, alveolar	1
	No significant findings	1
	Autolysis	1
Lymph node, mesenteric		**1**
	Autolysis	1
Parathyroid		**1**
	Autolysis	1
Spleen		**2**
	Autolysis	2

		Cont.
Thyroid		**1**
	Autolysis	1
Trachea		**2**
	No significant findings	1
	Autolysis	1
Urinary bladder		**1**
	Autolysis	1

Puma concolor (n=4)

Adipose tissue		**1**
	No significant findings	1
Adrenal glands		**3**
	Adrenalitis, mixed (lymphocytic, neutrophilic)	1
	Metazoan, nematode, larva migrans, medullae	1
	Vacuolar degeneration, cortex	1
	No significant findings	1
Brain		**2**
	No significant findings	2
Cerebellum		**1**
	No significant findings	1
Diaphragm		**2**
	No significant findings	2
Epiglottis		**1**
	No significant findings	1
Esophagus		**3**
	No significant findings	3
Heart		**3**
	No significant findings	3
Kidney		**4**
	Brownish pigment, glomeruli	1
	Mineralization, tubular, medullae	1
	Nephritis, interstitial, mononuclear (lymphocytic)	1
	Autolysis	2
Large intestine		**1**
	No significant findings	1
Larynx		**1**
	No significant findings	1
Liver		**4**
	Degeneration, steatosis, macrogoticular, hepatocyte	1
	No significant findings	1
	Autolysis	3
Lungs		**4**
	Edema, perivascular	4
	Hemorrhage, alveolar	4
	Edema, alveolar	3
	Edema, subpleural	2
	Hemorrhage, subpleural	2
	Metazoan, nematode, larvae, adult/L1 and eggs, alveolar lumen/septae, intravascular	2
	Pneumonia, granulomatous by lungworm	2
	Bullae, alveolar	1
	Bullae, subpleural	1
	Congestion, capillary beds	1
	Endoarteritis, granulocytic (neutrophilic), acute	1
	Hemorrhage, perivascular	1
	Hyperinsuflation, alveolar	1
Lymph node		**4**
	Paracortical lymphoid reactive hyperplasia	2

Cont.

No significant findings	1
Autolysis	2
Ovary	**1**
No significant findings	1
Pancreas	**1**
No significant findings	1
Autolysis	1
Salivary gland	**1**
No significant findings	1
Skeletal muscle	**3**
Metazoan, nematode, larvae, adult, subcutaneous with minimal granulocitic infiltrate	1
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
No significant findings	1
Autolysis	1
Skin	**3**
No significant findings	3
Small intestine	**4**
Enteritis, proliferative and fibrosing, eosinophilic with intralesional nematodes	2
Enteritis, mixed (lymphocytic, eosinophilic) by acanthocephala larvae	1
Leiomyositis, mixed (eosinophilic, histiocytic)	1
MALT hyperplasia	1
Metazoan, cestode, larvae, adult, lumen, no inflammation	1
Metazoan, nematode, ascarid, larvae, adult, lumen	1
Metazoan, nematode, undetermined, larvae, adult, lumen	1
Autolysis	2
Spleen	**4**
Hemosiderosis	1
White pulp depletion	1
No significant findings	1
Autolysis	4
Stomach	**4**
Gastritis, proliferative and fibrosing, eosinophilic with intralesional nematodes	3
No significant findings	1
Autolysis	2
Testicle	**1**
Azoospermia	1
Tongue	**4**
Glossitis, internal, mixed (neutrophilic, histiocytic, lymphocytic)	1
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
No significant findings	2
Autolysis	1
Trachea	**3**
No significant findings	3
Urinary bladder	**3**
No significant findings	1
Autolysis	2
Uterus	**1**
No significant findings	1

***Puma yagouaroundi* (n=12)**	
Adrenal glands	**4**
Adrenalitis, cortical, mononuclear (lymphocytic)	1
Adrenalitis, cortical, mononuclear (lymphocytic, histiocytic)	1
Cortical hemorrhage	1

		Cont.
	Hemorrhage, pericapsular	1
	Vacuolar degeneration, cortex, reticular	1
	Autolysis	1
Brain		**2**
	No significant findings	1
	Autolysis	1
Carotid		**1**
	No significant findings	1
Cerebellum		**1**
	No significant findings	1
Diaphragm		**1**
	Degeneration, myofiber	1
	Necrosis, myofiber	1
Epididymis		**1**
	Epididymitis, mononuclear (lymphocytic, plasmacytic)	1
Esophagus		**5**
	No significant findings	5
	Autolysis	1
Heart		**8**
	No significant findings	6
	Autolysis	2
Jugular		**1**
	No significant findings	1
Kidney		**7**
	Nephritis, interstitial, mononuclear (lymphocytic, plasmacytic)	1
	No significant findings	1
	Autolysis	6
Large intestine		**6**
	Colitis, follicular	1
	MALT hyperplasia	1
	No significant findings	2
	Autolysis	3
Liver		**7**
	Degeneration, hydropic, hepatocyte	1
	Degeneration, steatosis, macrogoticular, hepatocyte	1
	Hemorrhage, lobular	1
	Hepatitis, lobular, mixed (neutrophilic, lymphocytic)	1
	No significant findings	2
	Autolysis	5
Lungs		**10**
	Edema, alveolar	7
	Artery, tunica media, hypertrophy	5
	Hemorrhage, alveolar	5
	Bullae, alveolar	3
	Edema, perivascular	3
	Metazoan, nematode, larvae, adult/L1 and eggs, alveolar lumen/septae, bronchial/vascular lumen	3
	Bullae, subpleural	2
	Hemorrhage, perivascular	2
	Pneumonia, granulomatous by lungworm	2
	Congestion, capillary beds	1
	Edema, subpleural	1
	Hyperinsuflation, alveolar	1
	No significant findings	1
	Autolysis	2
Lymph node		**4**
	Paracortical lymphoid reactive hyperplasia	1

	Cont.
No significant findings	1
Autolysis	2
Pancreas	**3**
No significant findings	1
Autolysis	2
Penis	**1**
Balanitis, mononuclear (lymphocytic)	1
Skeletal muscle	**4**
Degeneration, myofiber	1
No significant findings	2
Autolysis	1
Small intestine	**7**
Leiomyositis, mixed (eosinophilic, histiocytic) by nematode larval migration	1
Metazoan, nematode, ascarid, larvae, adult, lumen	1
No significant findings	3
Autolysis	4
Spleen	**7**
No significant findings	4
Autolysis	4
Stomach	**5**
Gastritis, proliferative and fibrosing, eosinophilic with intralesional nematodes	2
No significant findings	1
Autolysis	4
Testicle	**4**
Sexual maturity	3
Azoospermia	1
Thymus	**2**
No significant findings	2
Tongue	**6**
Hemorrhage, muscular layer	1
No significant findings	3
Autolysis	2
Trachea	**5**
No significant findings	5
Urinary bladder	**6**
Leukocytic infiltrate, perivascular, mononuclear (lymphocytic)	1
No significant findings	2
Autolysis	3

Mephitidae

Table 7. General information of mephitids.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-229	*Conepatus semistriatus*	Female	Adult	1,72	Good	2018	May	Dry	MS	Nova Alvorada do Sul	Cerrado

Figure 6. Spatial distribution, and main characteristics of mephitids.
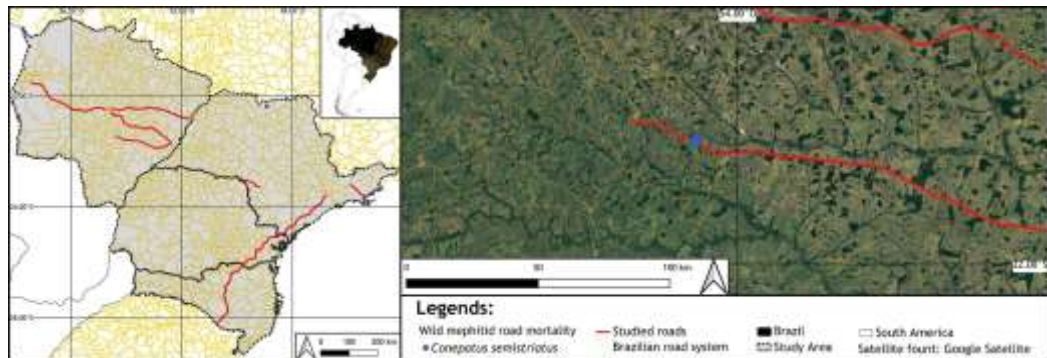
Table 8. Microscopic findings in mephitids. Striped Hog-nosed Skunk (*Conepatus semistriatus*, n=1).

Species, microscopic findings	Cases
***Conepatus semistriatus* (n=1)**	
Esophagus	**1**
No significant findings	1
Heart	**1**
Hemorrhage, myocardial	1
Myocarditis, mononuclear (lymphocytic, histiocytic)	1
Kidney	**1**
Proteinosis, tubular	1
Liver	**1**
Necrosis/apoptosis, hepatocyte, single cell	1
Lungs	**1**
Hemorrhage, alveolar	1
Hemorrhage, perivascular	1
Hemosiderosis	1
Pneumonia, interstitial, mononuclear (lymphocytic)	1
Lymph node	**1**
Paracortical lymphoid reactive hyperplasia	1
Skeletal muscle	**1**
Degeneration, myofiber	1
Necrosis, myofiber	1
Small intestine	**1**
Enteritis, granulomatous by nematodes	1
Spleen	**1**
Extramedular hematopoiesis	1
Stomach	**1**
No significant findings	1
Thymus	**1**
No significant findings	1
Tongue	**1**
Glossitis, internal, mixed (lymphocytic, neutrophilic)	1
Trachea	**1**
No significant findings	1
Urinary bladder	**1**
No significant findings	1

<u>Mustelidae</u>

Table 9. General information of Brazilian mustelids included in this study.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-012	*Galictis cuja*	Male	Juvenile	0,6	Regular	2016	February	Rain	SP	Miracatu	Atlantic forest
RK-035	*Lontra longicaudis*	Male	Adult	11	Good	2017	August	Dry	SP	Paraibuna	Atlantic forest
RK-057	*Galictis cuja*	Male	Adult	3,2	Good	2017	October	Rain	SP	Jambeiro	Atlantic forest
RK-261	*Galictis cuja*	Male	Adult	1,175	Good	2018	September	Dry	SP	São José dos Campos	Atlantic forest
RK-284	*Galictis cuja*	Male	Adult	1,3	Good	2018	October	Rain	SP	Ipaussu	Cerrado
RK-295	*Lontra longicaudis*	Female	Adult	6,15	Good	2018	August	Dry	MS	Corumbá	Pantanal
RK-326	*Eira barbara*	Male	Adult	6,85	Good	2019	May	Dry	SC	Unknown	Atlantic forest
RK-330	*Lontra longicaudis*	Male	Adult	6,08	Good	2019	May	Dry	SC	Papanduva	Atlantic forest
RK-341	*Galictis cuja*	Male	Adult	1,46	Good	2017	October	Rain	SC	Papanduva	Atlantic forest
RK-342	*Galictis cuja*	Male	Adult	1,32	Good	2018	October	Rain	PR	Campo do Tenente	Atlantic forest
RK-373	*Lontra longicaudis*	Male	Adult	5,62	Good	2019	May	Dry	SC	Unknown	Atlantic forest
RK-426	*Galictis cuja*	Male	Adult	1,42	Good	2019	November	Rain	SP	Jambeiro	Atlantic forest
RK-430	*Galictis cuja*	Male	Adult	1,45	Good	2019	October	Rain	SP	Paraibuna	Atlantic forest
RK-445	*Lontra longicaudis*	Female	Adult	-	Good	2019	December	Rain	MS	Corumbá	Pantanal

Figure 7. Spatial distribution, and main characteristics of mustelids included in this study.
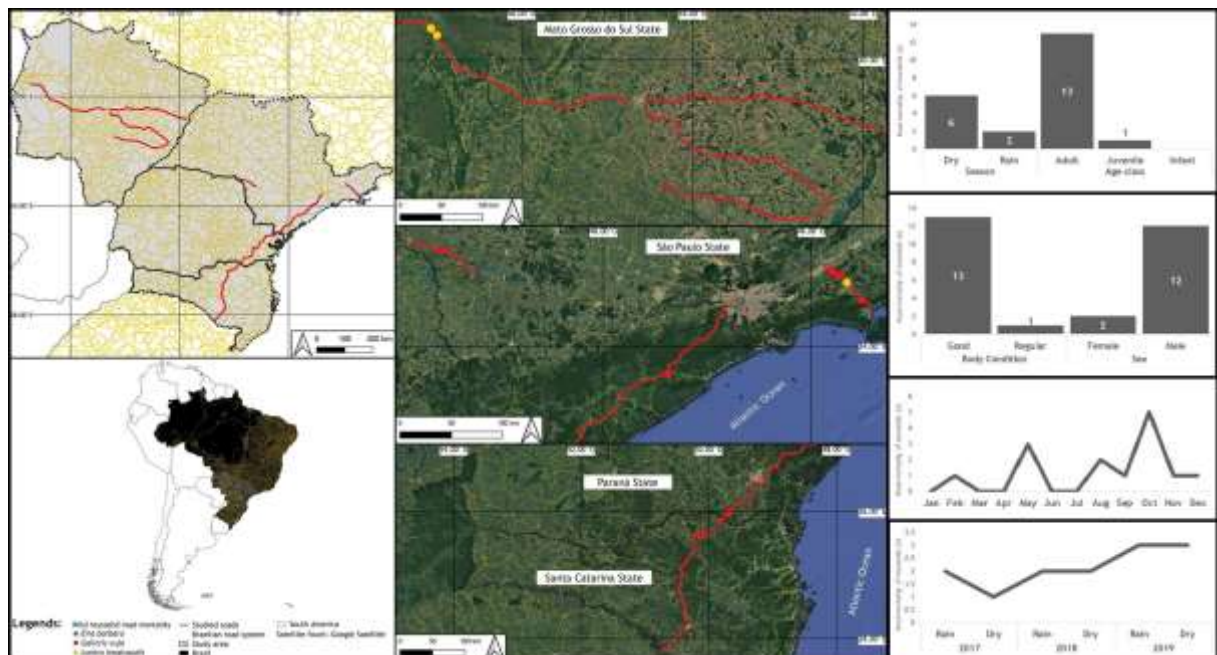
Table 10. Gross findings non-associated with trauma in mustelids.

Species, gross finding	Cases
***Eira barbara* (n=1)**	
Mesenteric lymph node megaly	100% (1/1)
***Galictis cuja* (n=8)**	
Mesenteric lymph node megaly	38% (3/8)
White pulp hyperplasia, spleen	13% (1/8)
Splenomegaly	13% (1/8)
Large intestine, endoparasitic infestation	13% (1/8)
Unilateral hydronephrosis (right) by Dyoctophyma sp.	13% (1/8)
***Lontra longicaudis* (n=5)**	
Mesenteric lymph node megaly	40% (2/5)
White pulp hyperplasia, spleen	20% (1/5)
Splenomegaly	20% (1/5)

Figure 8. Main gross findings in mustelids

RK-261. *Galictis cuja*. Male adult. Kidney. Hydronephrosis by giant kidney worm. *Dyoctophyma renale.*

RK-261. *Galictis cuja*. Male adult. Kidney. Hydronephrosis by giant kidney worm. *Dyoctophyma renale.*

RK-261. *Galictis cuja*. Male adult. Kidney. Hydronephrosis by giant kidney worm. *Dyoctophyma renale.*

RK-261. *Galictis cuja*. Male adult. Kidney. Hydronephrosis by giant kidney worm. *Dyoctophyma renale.*

Table 11. Microscopic findings in mustelids. Tayra (*Eira barbara*, n=2), lesser grison (*Galictis cuja*, n=8), neotropical river otter (*Lontra longicaudis*, n=5).

Species, microscopic findings	Cases
***Eira barbara* (n=2)**	
Adipose tissue	**1**
No significant findings	1
Heart	**2**
Degeneration, cardiomyocyte	1
Myocarditis, pyogranulomatous (histiocytic, neutrophilic) by protozoa	1
No significant findings	1
Kidney	**2**
No significant findings	1
Autolysis	1
Liver	**2**
Degeneration, hydropic, hepatocyte	1
Autolysis	2
Lungs	**1**
Edema, alveolar	1
Edema, perivascular	1
Autolysis	1
Lymph node	**1**
Autolysis	1
Lymph node, mesenteric	**1**
Lymphadenitis, granulocytic (neutrophilic)	1
Autolysis	1
Small intestine	**1**
Autolysis	1
Spleen	**2**
Autolysis	2
Stomach	**1**
Autolysis	1
Tongue	**1**
No significant findings	1
Trachea	**1**
No significant findings	1
***Galictis cuja* (n=8)**	
Adrenal glands	**4**
No significant findings	4
Brain	**1**
Autolysis	1
Carotid	**5**
No significant findings	5
Diaphragm	**4**
Degeneration, myofiber	1
Necrosis, myofiber	1
No significant findings	3
Esophagus	**5**
Lumen, nervous parenchyma	1
No significant findings	4
Gland	**1**
No significant findings	1
Heart	**8**
No significant findings	7
Autolysis	1

Cont.

Jugular **4**

No significant findings	4

Kidney **8**

Mineralization, tubular, medullae	1
Nephritis, interstitial, mononuclear (lymphocytic, histiocytic)	1
No significant findings	1
Autolysis	5

Large intestine **2**

No significant findings	1
Autolysis	1

Liver **8**

Degeneration, steatosis, macrogoticular, hepatocyte	2
Degeneration, hydropic, hepatocyte	1
Hepatitis, lobular, granulocytic (neutrophilic)	1
Hepatitis, lobular, mixed (neutrophilic, lymphocytic)	1
Hepatitis, lobular, mononuclear (lymphocytic)	1
Metazoan, trematode, larvae, adult, intraductal	1
No significant findings	2
Autolysis	4

Lungs **8**

Metazoan, nematode, larvae, adult/L1, alveolar lumen	7
Edema, alveolar	6
Hemorrhage, alveolar	6
Edema, perivascular	5
Hemorrhage, perivascular	3
Hyperinsuflation, alveolar	2
Artery, tunica media, hypertrophy	1
Hemosiderosis	1
Leukocyte infiltration, peribronchial, granulocytic (eosinophylic)	1
Leukocyte infiltration, perivascular, mononuclear (lymphocytic)	1
Mineralization, cartilage	1
Pneumonia, granulocytic (eosinophilic)	1
Pneumonia, granulocytic (neutrophilic)	1
Autolysis	2

Lymph node **3**

Paracortical lymphoid reactive hyperplasia	1
No significant findings	2
Autolysis	1

Lymph node, cervical **1**

Autolysis	1

Salivary gland **1**

No significant findings	1

Skeletal muscle **2**

Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
No significant findings	1

Skin **1**

Autolysis	1

Small intestine **5**

Metazoan, nematode, larvae, adult, lumen	1
No significant findings	2
Autolysis	3

Spleen **6**

Extramedular hematopoiesis	2
Hemosiderosis	1
White pulp hyperplasia	1

		Cont.
	Autolysis, advanced	3
Stomach		**2**
	No significant findings	2
Testicle		**6**
	Sexual maturity	6
Thymus		**1**
	No significant findings	1
Tongue		**3**
	Glossitis, internal, granulocytic (neutrophilic)	1
	Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
	No significant findings	1
Trachea		**6**
	No significant findings	6
Urinary bladder		**2**
	No significant findings	2

Lontra longicaudis (n=5)

Adrenal glands		**1**
	Adrenalitis, mononuclear (lymphocytic, histiocytic)	1
	Hemorrhage, cortical, fascicular	1
	Hyalinization, cortex	1
	Single cell necrosis/apoptosis, cortex, reticular	1
Brain		**1**
	Hemorrhage, neuroparenchyma	1
Carotid		**2**
	No significant findings	2
Diaphragm		**1**
	Degeneration, myofiber	1
	Protozoa, sarcocystid, intrasarcolemal, no inflammation	1
Esophagus		**2**
	Esophagitis, granulocytic (eosinophilic)	1
	Hemorrhage, periserosal	1
	No significant findings	1
Heart		**5**
	Hemorrhage, myocardial	1
	Leukocytic infiltrate, myocardial, mononuclear (lymphocytic)	1
	Myocarditis, mononuclear (lymphocytic, histiocytic)	1
	No significant findings	3
	Autolysis	1
Kidney		**3**
	Hemorrhage, cortical	1
	No significant findings	2
Large intestine		**1**
	Colitis, mixed (eosinophilic, lymphocytic)	1
Liver		**4**
	Ductular reaction	1
	Hepatitis, lobular, mononuclear (lymphocytic, histiocytic)	1
	Hepatitis, portal, mononuclear (lymphocytic)	1
	Leukocyte infiltration, portal, mononuclear (lymphocytic, plasmacytic)	1
	No significant findings	1
	Autolysis	1
Lungs		**4**
	Edema, alveolar	4
	Hemorrhage, alveolar	4
	Edema, perivascular	3
	Congestion, capillary beds	2

	Cont.
Hemorrhage, perivascular	2
Anthracosis	1
BALT hyperplasia	1
Bronchitis, mononuclear (lymphocytic)	1
Hemorrhage, bronchus	1
Hemorrhage, subpleural	1
Leukocyte infiltration, perivascular, mononuclear (lymphycytic)	1
Metazoan, nematode, larvae, adult, alveolar lumen, no inflammation	1
Mineralization, cartilage	1
Pneumonia, interstitial, granulocytic (eosinophilic)	1
Lymph node, mesenteric	**2**
Hemorrhage, sinusal	1
Paracortical lymphoid reactive hyperplasia	1
Ovary	**1**
No significant findings	1
Pancreas	**2**
No significant findings	2
Skeletal muscle, cervical	**1**
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
Small intestine	**2**
Enteritis, mixed (lymphocytic, eosinophilic)	1
Enteritis, mononuclear (lymphocytic, histiocytic)	1
MALT hyperplasia	1
Spleen	**4**
Extramedular hematopoiesis	1
Hemosiderosis	2
Necrosis, centrifollicular	1
White pulp hyperplasia	4
Stomach	**2**
No significant findings	2
Testicle	**2**
Sexual maturity	2
Trachea	**4**
Hemorrhage, mucosa	1
No significant findings	3
Urinary bladder	**3**
No significant findings	2
Autolysis, advanced	1
Uterus	**1**
No significant findings	1

Procyonidae

Table 12. General information of Brazilian procyonids included in this study.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-073	*Nasua nasua*	Female	Adult	3,51	Good	2017	December	Rain	MS	Campo Grande	Cerrado
RK-198	*Nasua nasua*	Female	Adult		Good	2018	February	Rain	MS	Aquidauana	Pantanal
RK-199	*Nasua nasua*	Male	Adult		Good	2018	March	Rain	MS	Corumbá	Pantanal
RK-314	*Nasua nasua*	Female	Adult	7,2	Good	2018	November	Rain	PR	Campina Grande do Sul	Atlantic forest
RK-316	*Nasua nasua*	Male	Adult	5,7	Good	2017	November	Rain	PR	Campina Grande do Sul	Atlantic forest

RK-329	*Nasua nasua*	Male	Adult	5,03	Good	2018	August	Dry	PR	Fazenda Rio Grande	Atlantic forest
RK-333	*Nasua nasua*	Male	Adult	7,02	Good	2018	March	Rain	SC	Itaiópolis	Atlantic forest
RK-392	*Nasua nasua*	Male	Adult	5,66	Good	2016	November	Rain	PR	Rio Negro	Atlantic forest
RK-402	*Nasua nasua*	Unknown	Adult		Good	2019	May	Dry	MS	Miranda	Pantanal
RK-405	*Nasua nasua*	Female	Adult	3,2	Good	2019	July	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-428	*Nasua nasua*	Male	Juvenile	2	Good	2019	October	Rain	SP	Caraguatatuba	Atlantic forest
RK-431	*Nasua nasua*	Male	Adult	3,1	Good	2019	November	Rain	SP	Paraibuna	Atlantic forest
RK-442	*Nasua nasua*	Female	Juvenile	2,2	Good	2019	December	Rain	MS	Anastácio	Cerrado
RK-010	*Procyon cancrivorus*	Female	Juvenile	5,6	Good	2016	January	Rain	SP	São Lourenço da Serra	Atlantic forest
RK-037	*Procyon cancrivorus*	Male	Adult	9,2	Good	2014	December	Rain	SP	Jacupiranga	Atlantic forest
RK-078	*Procyon cancrivorus*	Male	Adult	9,6	Good	2017	December	Rain	MS	Miranda	Pantanal
RK-080	*Procyon cancrivorus*	Female	Adult	9	Good	2017	December	Rain	MS	Aquidauana	Pantanal
RK-085	*Procyon cancrivorus*	Male	Adult	8,1	Good	2017	April	Dry	SP	Juquitiba	Atlantic forest
RK-179	*Procyon cancrivorus*	Male	Adult	8,2	Good	2018	January	Rain	SP	Caraguatatuba	Atlantic forest
RK-208	*Procyon cancrivorus*	Male	Juvenile	6,5	Good	2017	December	Rain	SP	Miracatu	Atlantic forest
RK-277	*Procyon cancrivorus*	Female	Adult	7	Good	2018	October	Rain	SP	Barra do Turvo	Atlantic forest
RK-279	*Procyon cancrivorus*	Male	Juvenile	6,3	Good	2018	March	Rain	PR	Campina Grande do Sul	Atlantic forest
RK-312	*Procyon cancrivorus*	Male	Juvenile	6,7	Good	2019	October	Rain	SP	Miracatu	Atlantic forest
RK-340	*Procyon cancrivorus*	Male	Adult	8,54	Good	2016	January	Rain	SC	Barra do Sul	Atlantic forest
RK-345	*Procyon cancrivorus*	Male	Juvenile	5,25	Good	2018	May	Dry	SC	São Francisco do Sul	Atlantic forest
RK-378	*Procyon cancrivorus*	Male	Adult	8,33	Good	2016	January	Rain	SC	Barra do Sul	Atlantic forest
RK-379	*Procyon cancrivorus*	Female	Adult	6,88	Good	2016	January	Rain	SC	São Francisco do Sul	Atlantic forest
RK-380	*Procyon cancrivorus*	Female	Adult	9,54	Good	2011	August	Dry	SC	São Francisco do Sul	Atlantic forest
RK-381	*Procyon cancrivorus*	Male	Adult	7,28	Good	2017	September	Dry	SC	São Francisco do Sul	Atlantic forest
RK-383	*Procyon cancrivorus*	Female	Adult	7,32	Good	2016	November	Rain	SC	São Francisco do Sul	Atlantic forest
RK-384	*Procyon cancrivorus*	Male	Adult	9,17	Good	2015	October	Rain	SC	Barra do Sul	Atlantic forest
RK-387	*Procyon cancrivorus*	Female	Adult	9,34	Good	2014	November	Rain	SC	Garuva	Atlantic forest
RK-410	*Procyon cancrivorus*	Female	Adult	6,6	Good	2019	June	Dry	SC	Barra do Turvo	Atlantic forest

Figure 9. Spatial distribution, and main characteristics of procyonids.
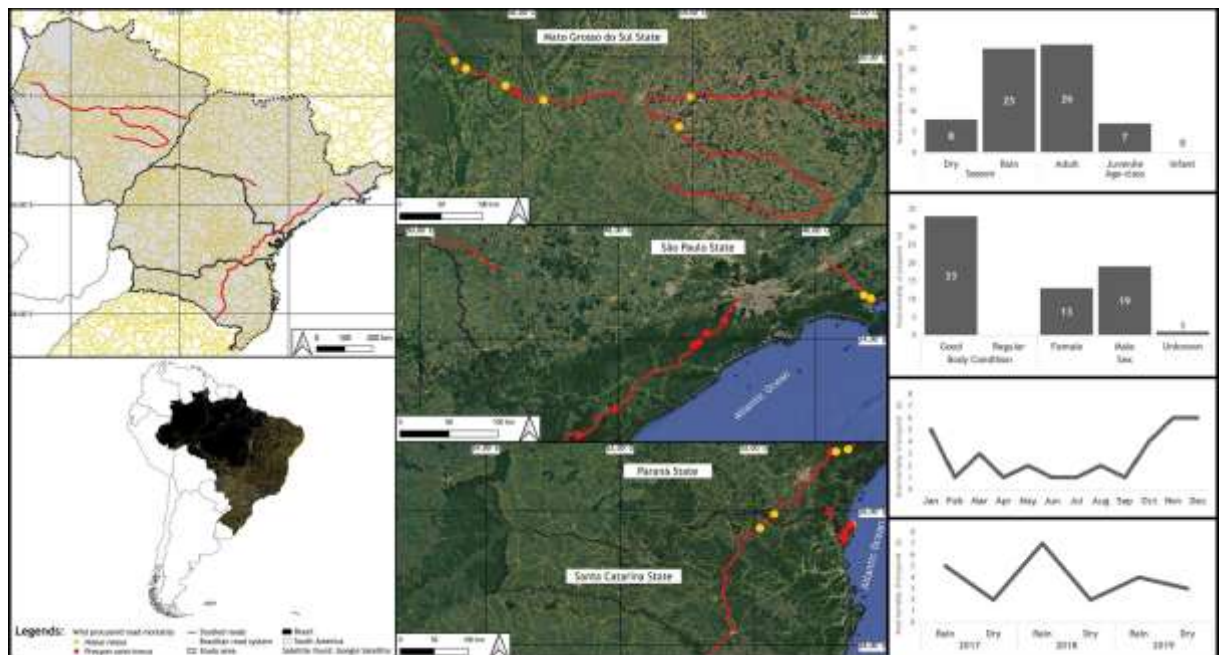
Table 13. Gross findings non-associated with in procyonids.

Species, gross finding	Cases
***Nasua nasua* (n=12)**	
White pulp hyperplasia, spleen	25% (3/12)
Tick infestation	25% (3/12)
Colitis by acanthocephala	17% (2/12)
Splenomegaly	17% (2/12)
Mesenteric lymph node megaly	17% (2/12)
Gallbladder, distended	8% (1/12)
Lactancy	8% (1/12)
Passive hepatic congestion	8% (1/12)
Pregnancy	8% (1/12)
Small intestine, endoparasitic infestation	8% (1/12)
Unilateral pyonephrosis	8% (1/12)
***Procyon cancrivorus* (n=20)**	
Mesenteric lymph node megaly	65% (13/20)
Tick infestation	45% (9/20)
Splenomegaly	20% (4/20)
Colitis by nematodes	20% (4/20)
Colitis by acanthocephala	5% (1/20)
White pulp hyperplasia, spleen	15% (3/20)
Small intestine, endoparasitic infestation	15% (3/20)
Gallbladder, distended	5% (1/20)
Lactancy	5% (1/20)
Pregnancy	5% (1/20)

Figure 10. Main gross findings.
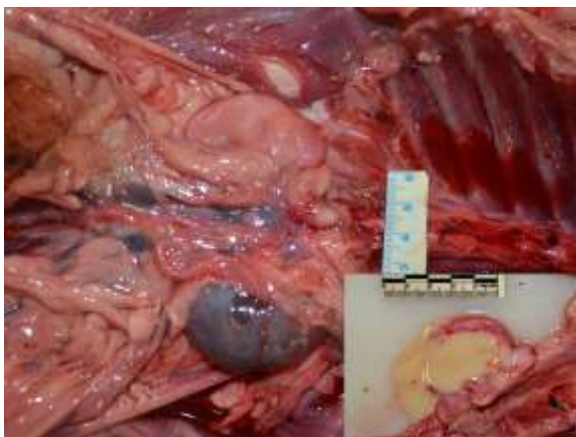
RK-314. *Nasua nasua*. Female, adult. Kidney. Unilateral pyonephrosis.

RK-279. *Procyon cancrivorus*. Male, Juvenile. Colon. Metazoan, Acanthocephala.

RK-279. *Procyon cancrivorus*. Male, Juvenile. Metazoan, Acanthocephala.

RK-410. *Procyon cancrivorus.* Female, adult. Mesenteric lymph node. Megaly.

Table 14.   Microscopic findings in procyonids. *Nasua nasua* (n=13), Procyon cancrivorus (n=20).

Species, microscopic findings	Cases
***Nasua nasua* (n=13)**	
Adrenal glands	**6**
Congestion, cortical, fascicular	1
Hemorrhage, pericapsular	1
Hemorrhage, periserosal	1
Leukocytic infiltrate, medullae, granulocytic (eosinophilic)	1
No significant findings	2
Autolysis	2
Brain	**3**
Edema, perivascular	1
Hemorrhage, leptomeningeal	1
Perivascular cuffing, mononuclear (lymphocytic)	1
Satellitosis	1
No significant findings	1
Carotid	**3**
No significant findings	3
Cerebellum	**2**
Hemorrhage, leptomeningeal	1
No significant findings	1
Diaphragm	**3**
Degeneration, myofiber	1
Myositis, mixed (neutrophilic, histiocytic)	1
Protozoa, sarcocystid, intrasarcolemal, no inflammation	1
Rupture, myofiber	1
No significant findings	1
Esophagus	**8**
Degeneration, myofiber	1
Esophagitis, mononuclear (lymphocytic)	1
Esophagitis, mononuclear (lymphocytic, histiocytic)	1
Hemorrhage, serosa	1
Protozoa, sarcocystid, intrasarcolemal, no inflammation	1
No significant findings	5
Heart	**10**
Degeneration, cardiomyocyte	2
Myocarditis, mononuclear (lymphocytic, histiocytic)	2

Cont.

Myocarditis, pyogranulomatous (histiocytic, neutrophilic) by protozoa	2
Metazoan, nematode, microfilaries, intravascular, no inflammation	1
No significant findings	3
Autolysis	2
Jugular	**2**
No significant findings	2
Kidney	**12**
Hemorrhage, cortex	2
Edema, perivascular	1
Hemorrhage, medullae	1
Hemorrhage, perivascular	1
Nephritis, interstitial, mononuclear (lymphocytic)	1
Pyelitis, mixed (eosinophylic, histiocytic) with intralesional nematode larvae	1
No significant findings	3
Autolysis	3
Large intestine	**3**
Colitis, transmural, ulcerative, mixed (eosinophilic, lymphocytic, histiocytic) by acanthocephala larvae	2
MALT hyperplasia	1
Metazoan, acanthocephala, larvae, lumen	1
Autolysis, advanced	1
Liver	**11**
Metazoan, nematode, microfilaries, portal, intravascular	2
Congestion, sinusoidal	1
Degeneration, hydropic, hepatocyte	1
Degeneration, steatosis, macrogoticular, hepatocyte	1
Degeneration, steatosis, microgoticular, hepatocyte	1
Hemosiderosis	1
Hepatitis, lobular, mixed (neutrophilic, lymphocytic)	1
Necrosis/apoptosis, hepatocyte, single cell	1
No significant findings	4
Autolysis	2
Lungs	**13**
Edema, alveolar	12
Hemorrhage, alveolar	10
Hyperinsuflation, alveolar	5
Edema, perivascular	4
BALT hyperplasia	3
Bullae, alveolar	3
Rupture, alveolar	2
Bronchopneumonia, fibrinous	1
Congestion, capillary beds	1
Edema, interlobar	1
Edema, subpleural	1
Emboli, bone narrow	1
Fetalization	1
Hemorrhage, interlobar	1
Hemorrhage, perivascular	1
Hemorrhage, subpleural	1
Hemosiderosis	1
Histiocytosis, alveolar	1
Metazoan, nematode, larvae, adult, alveolar lumen, no inflammation	1
Pneumonia, granulocytic (neutrophilic)	1
Pneumonia, interstitial, granulocytic (neutrophilic)	1
Autolysis	2

Cont.

Lymph node **4**

Extramedular hematopoiesis	1
Paracortical lymphoid reactive hyperplasia	1
Autolysis	1

Lymph node, cervical **3**

Paracortical lymphoid reactive hyperplasia	1
No significant findings	1
Autolysis	1

Lymph node, mesenteric **2**

Hemorrhage, sinusal	1
Lymphadenitis, granulocytic (neutrophilic)	1
Paracortical lymphoid reactive hyperplasia	1
No significant findings	1

Nostril **1**

No significant findings	1

Ovary **1**

No significant findings	1

Pancreas **7**

Metazoan, nematode, larvae, adult, duct	1
No significant findings	6
Autolysis	1

Salivary gland **3**

No significant findings	2
Autolysis	1

Skeletal muscle **3**

No significant findings	1
Autolysis	2

Skeletal muscle, cervical **2**

Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	2
Degeneration, myofiber	1
Myositis, mixed (neutrophilic, histiocytic, lymphocytic)	1

Skin **1**

No significant findings	1

Small intestine **8**

Enteritis, granulocytic (neutrophilic)	1
Enteritis, mixed (lymphocytic, histiocytic, eosinophilic) by nematodes	1
Leiomyositis, granulomatous (eosinophylic, histiocytic)	1
Metazoan, nematode, larvae, adult, fragments, lumen	1
No significant findings	2
Autolysis	3

Spleen **11**

White pulp hyperplasia	5
Hemorrhage, red pulp	1
Hemosiderosis	1
No significant findings	1
Autolysis	5

Stomach **8**

Gastritis, lamina propria, mixed (neutrophilic, lymphocytic, histiocytic)	2
Gastritis, mixed (eosinophylic, lymphocytic)	1
Gastritis-leiomyositis, granulomatous by nematode larvae	1
Leukocyte infiltrate, periganglionar, mononuclear (lymphocytic)	1
No significant findings	4

Testicle **4**

Sexual maturity	3
Azoospermia	1

Tongue **7**

Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	6

Glossitis, granulomatous by foreign body	2
Glossitis, mononuclear (histiocytic, lymphocytic)	2
Degeneration, ballooning, Keratinocytes,	1
Glossitis, internal, mixed (neutrophilic, histiocytic, lymphocytic)	1
Metazoan, nematode, Capillaria, larvae, adult and eggs, epithelium	1
Trachea	**9**
BALT hyperplasia	1
Hemorrhage, serosa	1
Tracheitis, mixed (eosinophilic, histiocytic)	1
Tracheitis, mononuclear (lymphocytic)	1
No significant findings	7
Urinary bladder	**10**
No significant findings	7
Autolysis	3
Uterus	**1**
No significant findings	1

Procyon cancrivorus (n=20)

Adrenal glands	**8**
Granuloma, parasitic, metazoan, nonidentifiable, pericapsular	1
Hemorrhage, pericapsular	1
Hyperplasia, cortex	1
No significant findings	4
Autolysis	3
Brain	**8**
Congestion	1
Edema, perivascular	1
Perivascular cuffing, mononuclear (lymphocytic)	1
Satellitosis	1
No significant findings	2
Autolysis	5
Carotid	**7**
No significant findings	7
Cerebellum	**2**
No significant findings	1
Autolysis	1
Colon	**1**
Colitis, granulocytic (eosinophilic) by metazoan parasite	1
Diaphragm	**4**
No significant findings	4
Epiglottis	**2**
No significant findings	2
Esophagus	**11**
Esophagitis, granulocytic (eosinophilic)	1
No significant findings	9
Autolysis	2
Heart	**18**
Myocarditis, granulocytic (neutrophilic)	1
Myocarditis, mononuclear (lymphocytic, histiocytic)	2
Myocarditis, pyogranulomatous (histiocytic, neutrophilic) by protozoa	1
No significant findings	11
Autolysis	3
Intestine	**1**
Autolysis	1
Jugular	**5**
No significant findings	5
Kidney	**15**
Dilatation, tubular	1

Cont.

Nephritis, interstitial, mononuclear (lymphocytic, histiocytic)	1
Nephritis, tubulointerstitial, mononuclear (lymphocytic, histiocytic)	1
Proteinosis, tubular	1
No significant findings	2
Autolysis	13
Large intestine	**5**
Leiomyositis, granulocytic (eosinophylic)	1
Metazoan, acanthocephala, larvae, lumen	2
No significant findings	1
Autolysis	2
Liver	**14**
Degeneration, hydropic, hepatocyte	1
Degeneration, steatosis, macrogoticular, hepatocyte	1
Focal granuloma, parenchyma	1
Hemorrhage, subcapsular	1
Hepatitis, lobular, mononuclear (lymphocytic)	1
Leukocyte infiltration, periductular, mononuclear (lymphocytic)	1
Pericholangitis, granulocytic (eosinophilic)	1
No significant findings	2
Autolysis	12
Lungs	**18**
Edema, alveolar	18
Edema, perivascular	14
Metazoan, nematode, larvae, adult, alveolar/bronchial/vascular lumen	10
Hemorrhage, alveolar	7
Autolysis, moderate	4
Congestion, capillary beds	4
Hemorrhage, perivascular	4
Pneumonia, granulomatous by lungworm	3
BALT hyperplasia	2
Bullae, alveolar	2
Hyperinsuflation, alveolar	2
Pneumonia, interstitial, granulocytic (eosinophilic)	2
Anthracosis	1
Artery, tunica media, hypertrophy	1
Bronchitis, mixed (eosinophilic, lymphocytic)	1
Bronchopneumonia, granulocytic (neutrophilic), acute	1
Bullae, subpleural	1
Emboli, bone narrow	1
Emboli, bone tissue	1
Endoarteritis, granulocytic (eosinophilic)	1
Hemorrhage, subpleural	1
Hemosiderosis	1
Leukocyte infiltration, perivascular, mononuclear (lymphocytic)	1
Pneumonia, bronchointerstitial, mixed (eosinophilic, lymphocytic, histiocytic) with MNGC	1
Pneumonia, granulocytic (neutrophilic)	1
Rupture, alveolar	1
Lymph node	**11**
Paracortical lymphoid reactive hyperplasia	2
Histiocytosis, sinusal	1
Leukocyte infiltration, medullar, granulocytic (eosinophilic)	1
Lymphadenitis, granulocytic (eosinophilic)	1
No significant findings	3
Autolysis	8
Lymph node, cervical	**1**
No significant findings	1

Cont.

Lymph node, mesenteric   **2**

Lymphadenitis, granulocytic (eosinophilic)	1
Autolysis	1

Ovary   **1**

No significant findings	1

Pad   **1**

No significant findings	1

Pancreas   **5**

No significant findings	3
Autolysis	2

Penis   **1**

No significant findings	1

Salivary gland   **2**

No significant findings	2

Skeletal muscle   **6**

Degeneration, myofiber	1
Hemorrhage, interstitial	1
Myositis, mixed (neutrophilic, histiocytic, lymphocytic)	1
Rupture, myofiber	1
No significant findings	2
Autolysis	1

Skin   **3**

Dermatitis, granulocytic (neutrophilic)	2
No significant findings	1
Autolysis	1

Small intestine   **11**

Enteritis, granulocytic (eosinophylic) by nematodes	1
Leiomyositis, granulomatous (eosinophylic, histiocytic) by trematode structures	1
No significant findings	4
Autolysis	8

Spine   **1**

No significant findings	1

Spleen   **14**

White pulp hyperplasia	3
Hemosiderosis	2
Hemorrhage, red pulp	1
Autolysis	10

Stomach   **5**

Parasitic granuloma	2
No significant findings	1
Autolysis	2

Testicle   **6**

Sexual maturity	4
Autolysis	2

Thymus   **1**

No significant findings	1

Tongue   **10**

Glossitis, mixed (neutrophilic, histiocytic)	1
No significant findings	8
Autolysis	1

Tonsil   **1**

No significant findings	1

Trachea   **15**

Metazoan, nematode, Capillaroid, larvae and eggs, submucosa	1
Tracheitis, granulocytic (eosinophilic) by nematode	1
Tracheitis, submucosal, mononuclear (lymphocytic)	1

		Cont.
	No significant findings	12
	Autolysis	1
Urinary bladder		**9**
	Cystitis, mixed (eosinophilic, lymphocytic, plasmacytic)	1
	No significant findings	7
	Autolysis	1

APPENDIX III

General information, gross and histopathological findings in armadillos

Table 1. General information of armadillos.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-386	*Cabassous tatouay*	Female	Adult	6,09	Good	2019	May	Dry	SC	Unknown	Atlantic forest
RK-129	*Cabassous unicinctus*	Female	Adult	2,6	Good	2017	September	Dry	MS	Campo Grande	Cerrado
RK-133	*Cabassous unicinctus*	Male	Adult	1,9	Good	2017	October	Rain	MS	Água Clara	Cerrado
RK-139	*Cabassous unicinctus*	Male	Juvenile	1,3	Good	2017	December	Rain	MS	Campo Grande	Cerrado
RK-324	*Cabassous unicinctus*	Female	Adult	-	Good	2019	February	Rain	MS	Campo Grande	Cerrado
RK-447	*Cabassous unicinctus*	Male	Adult	-	Good	2019	December	Rain	MS	Campo Grande	Cerrado
RK-014	*Dasypus novemcinctus*	Male	Adult	3,82	Good	2014	April	Dry	SP	Miracatu	Atlantic forest
RK-017	*Dasypus novemcinctus*	Male	Juvenile	3,2	Good	2014	November	Rain	SP	Registro	Atlantic forest
RK-036	*Dasypus novemcinctus*	Female	Juvenile	1,8	Regular	2017	March	Rain	SP	Paraibuna	Atlantic forest
RK-040	*Dasypus novemcinctus*	Female	Juvenile	2,7	Regular	2017	December	Rain	SP	Paraíbuna	Atlantic forest
RK-059	*Dasypus novemcinctus*	Male	Juvenile	3,2	Good	2017	October	Rain	SP	Caraguatatuba	Atlantic forest
RK-102	*Dasypus novemcinctus*	Male	Adult	-	Good	2017	March	Rain	MS	Terenos	Cerrado
RK-108	*Dasypus novemcinctus*	Male	Adult	-	Good	2017	May	Dry	MS	Terenos	Cerrado
RK-111	*Dasypus novemcinctus*	Female	Adult	3,7	Good	2017	May	Dry	MS	Nova Andradina	Cerrado
RK-181	*Dasypus novemcinctus*	Male	Adult	4,1	Regular	2018	January	Rain	MS	Terenos	Cerrado
RK-213	*Dasypus novemcinctus*	Female	Adult	4,3	Good	2018	June	Dry	MS	Aquidauana	Pantanal
RK-221	*Dasypus novemcinctus*	Male	Juvenile	2,98	Good	2018	May	Dry	MS	Aquidauana	Pantanal
RK-222	*Dasypus novemcinctus*	Male	Adult	3,88	Good	2018	April	Dry	MS	Anastácio	Cerrado
RK-223	*Dasypus novemcinctus*	Female	Adult	3,7	Good	2018	June	Dry	MS	Terenos	Cerrado
RK-252	*Dasypus novemcinctus*	Male	Adult	-	Good	2018	August	Dry	SP	Ipaussu	Cerrado
RK-282	*Dasypus novemcinctus*	Male	Adult	3,7	Good	2018	December	Rain	SP	Bernardino de Campos	Cerrado
RK-287	*Dasypus novemcinctus*	Female	Adult	4,7	Good	2018	October	Rain	MS	Jaraguari	Cerrado
RK-296	*Dasypus novemcinctus*	Male	Adult	4,6	Good	2018	October	Rain	SP	Jambeiro	Atlantic forest
RK-304	*Dasypus novemcinctus*	Male	Adult	3,8	Good	2018	October	Rain	SP	Paraibuna	Atlantic forest
RK-305	*Dasypus novemcinctus*	Female	Adult	3,7	Good	2018	November	Rain	SP	Jambeiro	Atlantic forest

RK-317	*Dasypus novemcinctus*	Female	Adult	4,8	Good	2018	November	Rain	SP	Itapecerica da Serra	Atlantic forest
RK-325	*Dasypus novemcinctus*	Unknown	Adult	-	Good	2019	March	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-395	*Dasypus novemcinctus*	Female	Adult	4,12	Good	2019	March	Rain	SC	Unknown	Atlantic forest
RK-413	*Dasypus novemcinctus*	Female	Adult	3,8	Good	2019	July	Dry	SP	Piraju	Cerrado
RK-418	*Dasypus novemcinctus*	Male	Juvenile	3,2	Good	2019	June	Dry	SP	Jambeiro	Atlantic forest
RK-439	*Dasypus novemcinctus*	Male	Adult	-	Good	2019	December	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-446	*Dasypus novemcinctus*	Male	Adult	-	Good	2019	December	Rain	MS	Miranda	Pantanal
RK-448	*Dasypus novemcinctus*	Male	Adult	-	Good	2019	December	Rain	MS	Miranda	Pantanal
RK-450	*Dasypus novemcinctus*	Male	Adult	-	Good	2019	December	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-061	*Euphractus sexcinctus*	Male	Adult	-	Good	2017	November	Rain	MS	Miranda	Pantanal
RK-100	*Euphractus sexcinctus*	Male	Adult	-	Good	2017	March	Rain	MS	Campo Grande	Cerrado
RK-104	*Euphractus sexcinctus*	Female	Adult	-	Good	2017	March	Rain	MS	Terenos	Cerrado
RK-114	*Euphractus sexcinctus*	Female	Adult	6,1	Good	2017	June	Dry	MS	Terenos	Cerrado
RK-136	*Euphractus sexcinctus*	Male	Adult	5,6	Good	2017	November	Rain	MS	Terenos	Cerrado
RK-137	*Euphractus sexcinctus*	Male	Adult	6,0	Good	2017	December	Rain	MS	Campo Grande	Cerrado
RK-140	*Euphractus sexcinctus*	Female	Adult	3,8	Good	2017	December	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-141	*Euphractus sexcinctus*	Female	Adult	5,4	Good	2017	December	Rain	MS	Aquidauana	Pantanal
RK-180	*Euphractus sexcinctus*	Male	Juvenile	3,5	Good	2018	January	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-182	*Euphractus sexcinctus*	Male	Adult	-	Good	2018	January	Rain	MS	Aquidauana	Pantanal
RK-183	*Euphractus sexcinctus*	Female	Adult	4,0	Good	2018	February	Rain	MS	Dois Irmãos do Buriti	Cerrado
RK-186	*Euphractus sexcinctus*	Male	Adult	5,5	Good	2018	February	Rain	MS	Água Clara	Cerrado
RK-188	*Euphractus sexcinctus*	Male	Adult	5,8	Regular	2018	February	Rain	MS	Anastácio	Cerrado
RK-214	*Euphractus sexcinctus*	Female	Adult	4,7	Regular	2018	June	Dry	MS	Anastácio	Cerrado
RK-224	*Euphractus sexcinctus*	Male	Juvenile	3,1	Good	2018	June	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-236	*Euphractus sexcinctus*	Male	Adult	-	Good	2018	July	Dry	MS	Terenos	Cerrado
RK-242	*Euphractus sexcinctus*	Male	Adult	5,27	Good	2018	July	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-436	*Euphractus sexcinctus*	Female	Adult	-	Good	2019	October	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-443	*Euphractus sexcinctus*	Male	Adult	-	Good	2019	December	Rain	MS	Santa Rita do Pardo	Cerrado
RK-451	*Euphractus sexcinctus*	Male	Adult	-	Good	2019	December	Rain	MS	Anastácio	Cerrado
RK-138	*Priodontes maximus*	Female	Adult	30,0	Good	2017	October	Rain	MS	Bataguassu	Cerrado
RK-403	*Priodontes maximus*	Male	Adult	28	Good	2019	June	Dry	MS	Nova Alvorada do Sul	Cerrado

Figure 1. Spatial distribution, and main characteristics of armadillos.
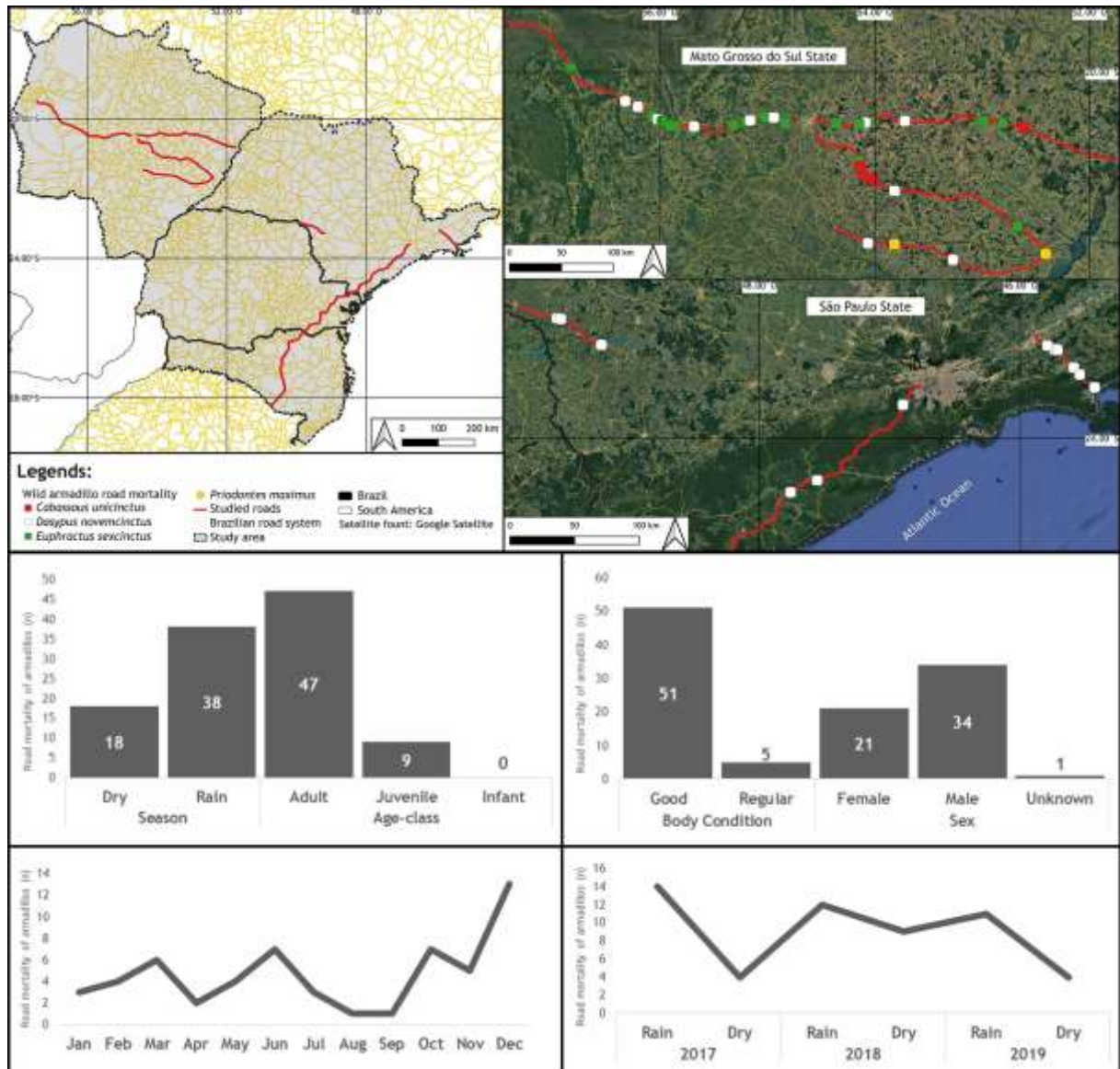
Table 2. Gross findings non-associated with trauma in armadillos.

Species, gross finding	Cases
***Cabassous unicinctus* (n=3)**	
Brain congestion	33% (1/3)
Splenomegaly	33% (1/3)
Passive hepatic congestion	33% (1/3)
***Dasypus novemcinctus* (n=25)**	
Mesenteric lymph node megaly	16% (4/25)
Tungiasis	16% (4/25)
Nodular pneumonia	13% (3/25)
Splenomegaly	8% (2/25)
Small intestine, endoparasitic infestation	8% (2/25)

	Cont.
Hepatic subcapsular nematode	4% (1/25)
Hepatitis, multifocal	4% (1/25)
Stomach, endoparasitic infestation	4% (1/25)
Tick infestation	4% (1/25)
***Euphractus sexcinctus* (n=20)**	
Splenomegaly	25% (5/20)
Nodular pneumonia	15% (3/20)
Splenic white pulp hyperplasia	10% (2/20)
Small intestine, endoparasitic infestation	10% (2/20)
Stomach, endoparasitic infestation	10% (2/20)
Hepatic granuloma	5% (1/20)
Lymph node megaly	5% (1/20)
***Priodontes maximus* (n=2)**	
Tick infestation	50% (1/2)

Figure 2. Main gross findings
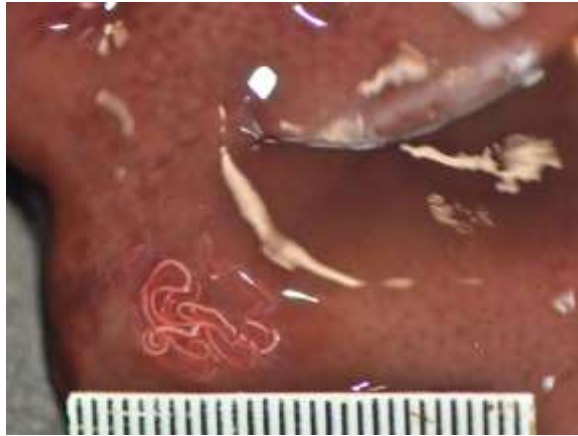
RK-282. *Dasypus novemcinctus*. Male, adult. Liver. Subcapsular larval migration. Metazoan, Nematode.

RK-296. *Dasypus novemcinctus*. Male, adult. Flea, genus, *Tunga* spp.

RK-304. *Dasypus novemcintus*. Male, adult. Flea, genus, *Tunga* spp.

Table 3. Microscopic findings in armadillos. Greater naked-tailed armadillo (*Cabassous tatouay*, n=1), naked-tailed armadillo (*Cabassous unicinctus*, n=5), long-nosed armadillo (*Dasypus novemcinctus*, n=28), six-banded Armadillo (*Euphractus sexcinctus*, n=21), giant Armadillo (*Priodontes maximus*, n=2).

Species, microscopic findings	N° cases
***Carassius tatouay* (n=1)**	
Heart	**1**
Autolysis	1
Kidney	**1**
Autolysis	1
Liver	**1**
Autolysis	1
Lungs	**1**
Autolysis	1
Spleen	**1**
Autolysis	1
Urinary bladder	**1**
Autolysis	1
***Carassius unicinctus* (n=5)**	
Adrenal glands	**2**
Hemorrhage, pericapsular	1
No significant findings	1
Brain	**1**
No significant findings	1
Carotid	**1**
No significant findings	1
Diaphragm	**1**
No significant findings	1
Esophagus	**4**
Hemorrhage, serosa	2
Degeneration, myofiber	1
Hemorrhage, submucosa	1
No significant findings	2
Heart	**3**
Hemorrhage, myocardial	1
No significant findings	2
Kidney	**3**
Hemorrhage, pericapsular	1
No significant findings	2
Large intestine	**2**
No significant findings	2
Liver	**4**
Degeneration, steatosis, microgoticular, hepatocyte	1
Hemorrhage, subcapsular/parenchymal	1
No significant findings	3
Lungs	**4**
Hemorrhage, alveolar	3
BALT hyperplasia	2
Congestion, capillary beds	2
Congestion, vascular	1
Edema, alveolar	1
Hemorrhage, bronchus	1

	Cont.
Hemorrhage, perivascular	1
Hemosiderosis	1
Hyperinsuflation, alveolar	1
Pneumonia, pyogranulomatous by adiaspores (Emmonsia)	1
Rupture, alveolar	1
Penis	**1**
No significant findings	1
Salivary gland	**2**
No significant findings	2
Skeletal muscle	**3**
Degeneration, myofiber	1
Rupture, myofiber	1
No significant findings	2
Small intestine	**4**
Protozoa, Eimeria, crypta	1
No significant findings	3
Spleen	**1**
White pulp hyperplasia	1
Stomach	**3**
Leukocyte infiltrate, lamina propria, mononuclear (lymphocytic, histiocytic)	1
No significant findings	2
Testicle	**1**
Azoospermia	1
Thymus	**1**
No significant findings	1
Thyroid	**1**
No significant findings	1
Tongue	**2**
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
No significant findings	1
Trachea	**2**
No significant findings	2

***Dasypus novemcinctus* (n=28)**	
Adrenal glands	**6**
Hemorrhage, pericapsular	1
No significant findings	4
Autolysis, advanced	1
Brain	**3**
Hemorrhage, leptomeningeal	1
Perivascular cuffing, mononuclear (lymphocytic)	1
No significant findings	2
Carotid	**1**
No significant findings	1
Cerebellum	**2**
No significant findings	2
Diaphragm	**4**
No significant findings	4
Epiglottis	**1**
No significant findings	1
Esophagus	**9**
Protozoa, sarcocystid, Intrasarcolemal, no inflammation	1
No significant findings	8
Heart	**21**
Myocarditis, mononuclear (lymphocytic, histiocytic)	2
Fibrosis, myocardium	1
Leukocytic infiltrate, perivascular, mononuclear (lymphocytic)	1

	Cont.
Metazoan, nematode, microfilaries, intravascular, no inflammation	1
No significant findings	15
Autolysis	3
Jugular	**1**
No significant findings	1
Kidney	**17**
Nephritis, interstitial, mononuclear (lymphocytic)	3
Leukocytic infiltrate, perivascular, mononuclear (lymphocytic)	2
Proteinosis, tubular	2
Proteinosis, tubular/glomerular	2
Hemorrhage, subcapsular	1
Proteinosis, glomerular	1
No significant findings	5
Autolysis	6
Large intestine	**2**
No significant findings	1
Autolysis	1
Liver	**18**
Hepatitis, granulomatous by metazoan	4
Hemorrhage, lobular	2
Hepatitis, lobular, mononuclear (lymphocytic, histiocytic)	2
Laceration, parenchyma	2
Cyst, lobular, cestode, hydatid	1
Degeneration, steatosis, microgoticular, hepatocyte	1
Ductular reaction, bile duct	1
Hemosiderosis	1
Hepatitis, lobular, granulocytic (neutrophilic)	1
Leukocyte infiltration, periductular, mononuclear (lymphocytic)	1
Leukocyte infiltration, sinusoids, mixed (neutrophilic, lymphocytic)	1
Metazoan, nematode, egg	1
Multinucleation, hepatocyte	1
Sinusoids, cocci	1
No significant findings	2
Autolysis	8
Lungs	**28**
Edema, alveolar	23
Hemorrhage, alveolar	21
Congestion, capillary beds	13
Hemorrhage, perivascular	11
Edema, perivascular	10
Congestion, vascular	9
Pneumonia, bronchointerstitial, subacute with MNGC	5
Bronchopneumonia, acute, granulocytic (neutrophilic)	4
Granuloma, parasitic	3
Hemosiderosis	3
Pneumonia, granulocytic (eosinophilic)	3
Pneumonia, interstitial, mixed (eosinophilic, lymphocytic)	3
Bronchitis, fibrinous, granulocytic (eosinophilic)	2
Edema, subpleural	2
Leukocyte infiltration, perivascular, mononuclear (lymphocytic, plasmacytic)	2
Metazoan, nematode, microfilaries, intravascular	2
Rupture, alveolar	2
Alveolitis, acute, granulocytic (neutrophilic)	1
Anthracosis	1
Bronchopneumonia, fibrinosuppurative	1
Bronchopneumonia, mixed (eosinophilic, histiocytic) by nematode larvae	1

Cont.

Bullae, subpleural	1
Emboli, hepatic tissue, hepatocytes	1
Hemorrhage, subpleural	1
Hyperinsuflation, alveolar	1
Metazoan, cestode, cyst	1
Metazoan, nematode, larvae, adult, bronchial lumen	1
Pleuritis, mononuclear (lymphocytic, histiocytic), chronic	1
Pneumonia, granulomatous	1
Pneumonia, interstitial, granulocytic (eosinophilic)	1
Autolysis	5
Lymph node	**5**
Paracortical lymphoid reactive hyperplasia	2
Hemosiderosis	1
Lymphadenitis, necrotizing	1
No significant findings	1
Autolysis	1
Lymph node, mesenteric	**1**
Hemosiderosis	1
Histiocytosis, sinusal	1
Paracortical lymphoid reactive hyperplasia	1
Lymph node, peribronchial	**1**
Paracortical lymphoid reactive hyperplasia	1
Ovary	**1**
No significant findings	1
Pancreas	**3**
Hemorrhage, periductal	1
No significant findings	2
Salivary gland	**6**
Hemorrhage, parenchyma	1
No significant findings	4
Autolysis, advanced	2
Skeletal muscle	**10**
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	4
Rupture, myofiber	1
No significant findings	5
Skin	**7**
Arthropod, Tunga, dermis, no inflammation	2
Orthokeratotic hyperkeratosis	1
No significant findings	4
Small intestine	**10**
Enteritis, granulocytic (eosinophylic)	1
Enteritis, mononuclear (lymphocytic, histiocytic)	1
MALT hyperplasia	1
No significant findings	4
Autolysis	3
Spleen	**12**
Hemosiderosis	3
Extramedular hematopoiesis	2
Histiocytosis, red pulp	2
White pulp depletion	2
Hemorrhage, red pulp	1
Plasmocitosis	1
Splenitis, granulocytic (neutrophilic)	1
Autolysis	2
Stomach	**10**
Gastritis, granulocytic (eosinophylic)	2
Parasitic granuloma, submucosa	1

		Cont.
	No significant findings	5
	Autolysis	3
Testicle		**9**
	Sexual maturity	8
	No significant findings	2
Thymus		**1**
	No significant findings	1
Thyroid		**1**
	No significant findings	1
Tongue		**16**
	Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	7
	Glossitis, internal, granulocytic (neutrophilic)	1
	Glossitis, internal, mixed (neutrophilic, histiocytic, lymphocytic)	1
	Glossitis, mononuclear (lymphocytic)	1
	No significant findings	8
Trachea		**9**
	Tracheitis, granulocytic (eosinophilic)	1
	No significant findings	8
Urinary bladder		**7**
	Cystitis, granulocytic (eosinophylic)	1
	Hemorrhage, mucosa	1
	No significant findings	5
Uterus		**1**
	No significant findings	1

Euphractus sexcinctus (n=21)

Adrenal glands		**4**
	Congestion, cortical, fascicular	1
	Hemorrhage, cortical, fascicular	1
	Hemorrhage, cortical, fascicular/reticular	1
	Hyperplasia, cortex	1
	Vacuolar degeneration, cortex, fascicular	1
	No significant findings	1
Brain		**2**
	Hemorrhage, leptomeningeal	1
	Hemorrhage, leptomeningeal/neuroparenchyma	1
	Perivascular cuffing, mononuclear (lymphocytic)	1
Diaphragm		**2**
	No significant findings	2
Esophagus		**6**
	Hemorrhage, serosa	1
	No significant findings	5
Heart		**8**
	Endocarditis, mononuclear (histiocytic)	1
	Hemorrhage	1
	No significant findings	6
Kidney		**10**
	Proteinosis, glomerular	3
	Proteinosis, tubular	3
	Proteinosis, tubular/glomerular	3
	Capsulitis, granulocytic (neutrophilic)	1
	Hemorrhage, subcapsular	1
	Nephritis, interstitial, mononuclear (lymphocytic)	1
	No significant findings	5
Large intestine		**5**
	MALT hyperplasia	1
	No significant findings	4

Cont.

Liver **8**

Degeneration, hydropic, hepatocyte	2
Ductular reaction, bile duct	2
Binucleation, hepatocyte	1
Hemorrhage, lobular	1
Hepatitis, interface, mixed (neutrophilic, lymphocytic)	1
Hepatitis, lobular, granulocytic (neutrophilic)	1
Hepatocytes, Intracytoplasmic glass like inclusion	1
Laceration, parenchyma	1
Leukocyte infiltration, sinusoids, mixed (neutrophilic, lymphocytic)	1
Pericholangitis, mixed (eosinophilic, lymphocytic)	1
No significant findings	5

Lungs **19**

Hemorrhage, alveolar	19
Edema, alveolar	17
Congestion, capillary beds	16
Edema, perivascular	15
Congestion, vascular	13
Hemorrhage, perivascular	13
BALT hyperplasia	11
Pneumonia, pyogranulomatous by adiaspores (Emmonsia)	9
Metaplasia, bone narrow	5
Histiocytosis, alveolar	3
Leukocyte infiltration, perivascular, mononuclear (lymphocytic, plasmacytic)	3
Pleuritis, mononuclear (lymphocytic, histiocytic), chronic	3
Fungus, adiaspores, Emmonsia, alveolar septae	2
Atelectasis	1
Bronchopneumonia, mixed (eosinophilic, histiocytic, lymphocytic) by nematode larvae (capillaroid egg)	1
Hemosiderosis	1
Leukocyte infiltration, perivascular, mixed (lymphocytic, plasmacytic, eosinophilic)	1
Leukocyte infiltration, perivascular, mononuclear (lymphocytic)	1
Metazoan, nematode, microfilaries, intravascular	1
Pneumonia, bronchointerstitial, subacute with MNGC	1
Pneumonia, granulomatous with MNGC	1
Pneumonia, interstitial, mononuclear (histiocytic)	1

Lymph node **3**

Paracortical lymphoid reactive hyperplasia	2
No significant findings	1

Lymph node, mesenteric **1**

Paracortical lymphoid reactive hyperplasia	1

Pancreas **6**

Hemorrhage, parenchyma	3
No significant findings	3

Salivary gland **5**

No significant findings	5

Skeletal muscle **4**

Degeneration, myofiber	1
Myositis, granulocytic (neutrophilic)	1
Myositis, mixed (neutrophilic, histiocytic, lymphocytic)	1
No significant findings	1

Skin **1**

Leukocyte infiltration, perivascular, dermis, mixed (neutrophilic, histiocytic)	1

Cont.

Small intestine	**8**
Enteritis, mononuclear (lymphocytic, histiocytic)	2
MALT hyperplasia	2
Protozoa, Eimeria, crypta	2
Enteritis, mixed (lymphocytic, eosinophilic)	1
Metazoan, cestode, larvae, lumen	1
Metazoan, undetermined	1
No significant findings	2
Autolysis	1
Spleen	**7**
Extramedular hematopoiesis	5
Hemorrhage, red pulp	3
Hemosiderosis	3
White pulp hyperplasia	2
Histiocytosis, red pulp	1
Leukocytic infiltrate, red pulp, granulocytic (eosinophilic)	1
Necrosis, centrifollicular	1
Autolysis	1
Stomach	**4**
Gastritis, granulocytic (neutrophilic)	2
Parasitic granuloma, submucosa	1
No significant findings	1
Testicle	**2**
Sexual maturity	1
No significant findings	1
Thymus	**3**
No significant findings	3
Thyroid	**1**
No significant findings	1
Tongue	**8**
Degeneration, myofiber	1
Glossitis, granulomatous by foreign body	1
Glossitis, internal, mixed (neutrophilic, histiocytic, lymphocytic)	1
Glossitis, mononuclear (histiocytic, lymphocytic)	1
No significant findings	5
Trachea	**7**
Hemorrhage, submucosa	1
Tracheitis, mixed (eosinophilic, neutrophilic, lymphocytic) by with nematodes (capillaroid eggs)	1
Tracheitis, submucosal, mixed (eosinophilic, lymphocytic)	1
No significant findings	4
Urinary bladder	**6**
No significant findings	6

***Priodontes maximus* (n=2)**	
Carotid	**1**
No significant findings	1
Heart	**2**
No significant findings	2
Kidney	**1**
Autolysis, moderate	1
Liver	**2**
Congestion, sinusoidal	1
Hemorrhage, lobular	1
Autolysis,	1
Lungs	**2**
Edema, alveolar	2

	Congestion, capillary beds	1
	Hemorrhage, alveolar	1
	Hemosiderosis	1
Lymph node		**1**
	Extramedular thrombopoiesis	1
	Paracortical lymphoid reactive hyperplasia	1
Salivary gland		**1**
	No significant findings	1
Skeletal muscle		**1**
	No significant findings	1
Spleen		**2**
	Hemorrhage, red pulp	1
	Autolysis, advanced	1
Stomach		**1**
	No significant findings	1
Testicle		**1**
	Sexual maturity	1
Tongue		**1**
	Glossitis, granulomatous by foreign body	1
Trachea		**2**
	Hemorrhage, serosa	1
	No significant findings	1

APPENDIX IV

General information, gross and histopathological findings in opossums.

Table 1. General information of opossums.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-019	*Didelphis aurita*	Male	Juvenile	1,25	Good	2014	January	Rain	SP	Miracatu	Atlantic forest
RK-023	*Didelphis aurita*	Male	Juvenile	1,14	Regular	2015	September	Dry	SP	Juquitiba	Atlantic forest
RK-031	*Didelphis aurita*	Male	Adult	2,2	Good	2015	September	Dry	SP	Juquitiba	Atlantic forest
RK-052	*Didelphis aurita*	Male	Juvenile	0,83	Regular	2017	February	Rain	SP	Paraibuna	Atlantic forest
RK-178	*Didelphis aurita*	Female	Juvenile	0,7	Good	2018	April	Dry	SP	Piracicaba	Atlantic forest
RK-239	*Didelphis albiventris*	Male	Juvenile	1,11	Good	2018	July	Dry	MS	Terenos	Cerrado
RK-249	*Didelphis aurita*	Male	Adult	2,5	Good	2018	April	Dry	SP	Paraibuna	Atlantic forest
RK-259	*Didelphis aurita*	Male	Adult	1	Good	2018	August	Dry	SP	Paraibuna	Atlantic forest
RK-260	*Didelphis aurita*	Male	Adult	2,2	Good	2018	September	Dry	SP	Jambeiro	Atlantic forest
RK-306	*Philander frenatus*	Male	Adult	0,24	Good	2019	January	Rain	SP	Paraibuna	Atlantic forest
RK-357	*Didelphis albiventris*	Male	Adult	2,43	Good	2014	June	Dry	SC	Joinville	Atlantic forest
RK-361	*Didelphis albiventris*	Male	Juvenile	1,59	Good	2007	July	Dry	RS	Osório	Atlantic forest
RK-382	*Didelphis albiventris*	Male	Adult	2,04	Good	2012	August	Dry	SC	São Francisco do Sul	Atlantic forest
RK-388	*Didelphis albiventris*	Male	Adult	2,14	Good	2007	June	Dry	SC	Laguna	Atlantic forest
RK-416	*Didelphis aurita*	Female	Adult	1,3	Good	2019	June	Dry	SP	Caraguatatuba	Atlantic forest
RK-420	*Didelphis aurita*	Female	Juvenile	1,1	Good	2019	September	Dry	SP	Paraibuna	Atlantic forest
RK-421	*Didelphis aurita*	Male	Juvenile	1,25	Good	2019	August	Dry	SP	Caraguatatuba	Atlantic forest
RK-422	*Didelphis aurita*	Male	Adult	1,8	Good	2019	September	Dry	SP	Caraguatatuba	Atlantic forest
RK-425	*Philander frenatus*	Male	Adult	0,25	Good	2019	July	Dry	SP	Paraibuna	Atlantic forest
RK-427	*Didelphis aurita*	Male	Juvenile	1,25	Good	2019	October	Rain	SP	Paraibuna	Atlantic forest
RK-432	*Didelphis aurita*	Male	Adult	1,9	Good	2019	October	Rain	SP	Caraguatatuba	Atlantic forest

Figure 1. Spatial distribution, and main characteristics of opossums.
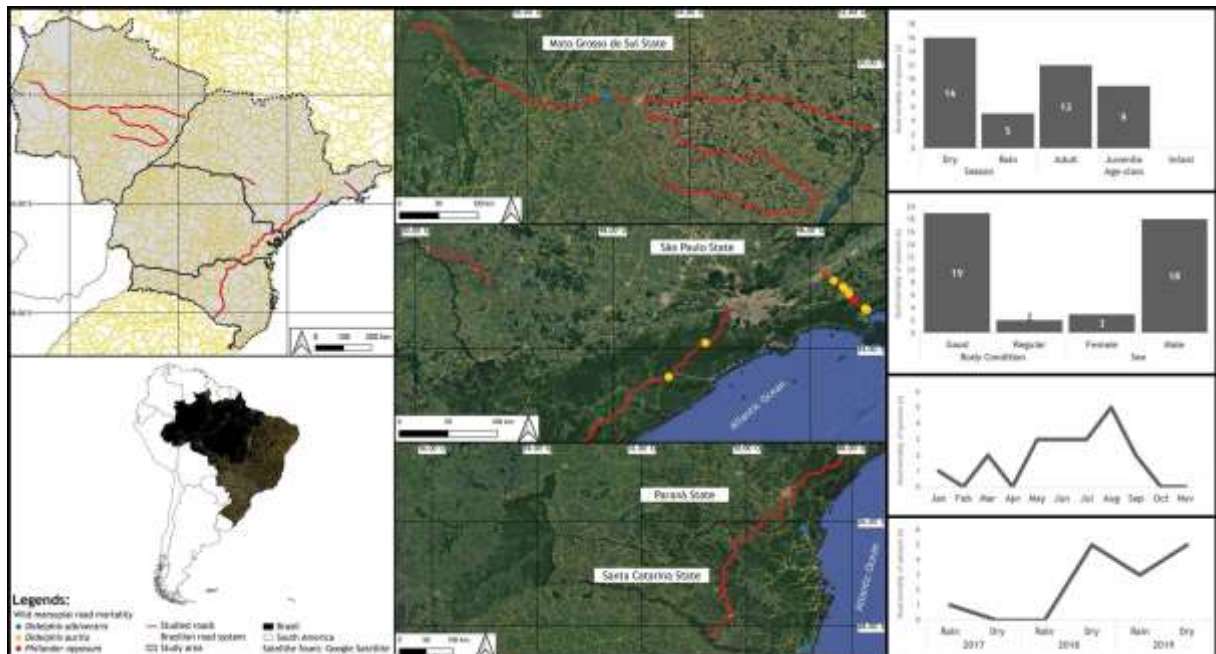
Table 2. Gross findings non associated with trauma in opossums.

Species, gross finding	Cases
Didelphis albiventris (n=5)	
Gallbladder, distended	20% (1/5)
Hepatic nematode migration	20% (1/5)
Small intestine, endoparasitic infestation	20% (1/5)
Didelphis aurita (n=14)	
Tick infestation	43% (6/14)
Small intestine, endoparasitic infestation	29% (4/14)
Splenomegaly	14% (2/14)
Gallbladder, distended	14% (2/14)
Lymph node megaly	14% (2/14)
Pregnancy	14% (2/14)
Consolidated fracture	7% (1/14)
Large intestine, endoparasitic infestation	7% (1/14)
Passive hepatic congestion	7% (1/14)
Pulmonary adherences	7% (1/14)
Philander frenatus (n=2)	
Splenomegaly	50% (1/2)

Figure 2. Main Gross findings in opossums.

RK-019. *Didelphis aurita*. Male, juvenile. Tick, Family: *Ixodidae*, genus: *Amblyomma*.

RK-259. *Didelphis aurita*. Male, adult. Intestine. Metazoan parasite. Cestode.

Table 3. Microscopic findings of opossums. White-eared Opossum (*Didelphis albiventris*, n=5), Big-eared Opossum (*Didelphis aurita*, n=14), gray four-eyed opossum (*Philander opossum*, n=2).

Species, microscopic findings	N° cases
***Didelphis albiventris* (n=5)**	
Carotid	**1**
No significant findings	1
Esophagus	**2**
No significant findings	2
Heart	**3**
Degeneration, cardiomyocyte	1
Necrosis, cardiomyocyte	1
Autolysis, advanced	1
Jugular	**1**
No significant findings	1
Large intestine	**1**
No significant findings	1
Liver	**1**
Degeneration, hydropic, hepatocyte	1
Lungs	**3**
Hemorrhage, alveolar	1
Metazoan, nematode, larvae, adult, alveolar lumen	1
Pneumonia, granulomatous by lungworm	1
Autolysis	2
Lymph node	**1**
No significant findings	1
Skeletal muscle	**1**
No significant findings	1
Small intestine	**2**
Metazoan, undetermined	1
No significant findings	1
Autolysis	1

Spleen	**2**
Hemorrhage, red pulp	1
Autolysis, advanced	1
Stomach	**1**
Gastritis, granulocytic (eosinophylic)	1
Testicle	**2**
Sexual maturity	1
Leydig cell hyperplasia	1
Tongue	**2**
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
Autolysis	1
Trachea	**3**
No significant findings	2
Autolysis	1
Urinary bladder	**1**
Autolysis	1

***Didelphis aurita* (n=14)**

Carotid	**1**
No significant findings	1
Diaphragm	**1**
Autolysis	1
Epiglottis	**2**
No significant findings	2
Esophagus	**8**
Hemorrhage, periserosal	1
No significant findings	7
Gland	**3**
No significant findings	3
Heart	**11**
Leukocytic infiltrate, mononuclear (lymphocytic)	1
No significant findings	6
Autolysis	4
Jugular	**2**
No significant findings	2
Kidney	**10**
Nephritis, interstitial, mononuclear (lymphocytic)	2
Nephritis, interstitial, mixed (lymphocytic, histiocytic, eosinophylic)	1
Autolysis	8
Liver	**12**
Degeneration, steatosis, macrogoticular, hepatocyte	3
Atrophy, hepatocyte	1
Degeneration, hydropic, hepatocyte	1
Hepatitis, lobular, granulocytic (neutrophilic)	1
Autolysis	9
Lungs	**13**
Edema, alveolar	7
Edema, perivascular	7
Hemorrhage, alveolar	4
Metazoan, nematode, larvae, adult, alveolar lumen, no inflammation	4
BALT hyperplasia	2
Hyperinsuflation, alveolar	2
Bullae, subpleural	1
Congestion, capillary beds	1
Edema, subpleural	1
Granuloma, parasitic	1

Cont.

	Mineralization, bronchial	1
	Autolysis	5
Lymph node		**3**
	Autolysis	3
Lymph node, mesenteric		**1**
	Autolysis	1
Salivary gland		**3**
	No significant findings	3
Skeletal muscle		**6**
	Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	3
	Rupture, myofiber	1
	No significant findings	3
	Autolysis	1
Skin		**2**
	No significant findings	1
	Autolysis	1
Small intestine		**2**
	Metazoan, undetermined	1
	Autolysis	2
Spleen		**5**
	Autolysis	5
Stomach		**4**
	Autolysis	4
Testicle		**6**
	Sexual maturity	3
	No significant findings	2
	Autolysis	2
Tongue		**6**
	Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1
	No significant findings	4
	Autolysis	2
Trachea		**9**
	Hemorrhage, serosa	1
	No significant findings	7
	Autolysis	1
Urinary bladder		**7**
	No significant findings	1
	Autolysis	6
Uterus		**1**
	No significant findings	1

Philander opossum (n=2)		
Carotid		**1**
	No significant findings	1
Esophagus		**2**
	No significant findings	2
Heart		**2**
	Myocarditis, granulocytic (neutrophilic)	1
	No significant findings	1
Jugular		**1**
	No significant findings	1
Kidney		**1**
	Mineralization, tubular, medullae	1
	Autolysis, advanced	1
Liver		**2**
	Degeneration, steatosis, microgoticular, hepatocyte	1
	No significant findings	1

		Cont.
Lungs		**2**
	Edema, alveolar	2
	Hemorrhage, alveolar	2
	Artery, tunica media, hypertrophy	1
	Congestion, capillary beds	1
	Edema, perivascular	1
Salivary gland		**1**
	No significant findings	1
Skeletal muscle		**1**
	No significant findings	1
Spleen		**2**
	No significant findings	1
	Autolysis, advanced	1
Stomach		**2**
	No significant findings	2
Testicle		**2**
	Sexual maturity	1
	No significant findings	1
Thyroid		**1**
	No significant findings	1
Tongue		**2**
	No significant findings	2
Trachea		**2**
	No significant findings	2
Urinary bladder		**1**
	No significant findings	1

APPENDIX V

General information, gross and histopathological findings in lagomorphs

Table 1. General information of lagomorphs.

Case	Specie	Sex	Age	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-315	*Lepus europaeus*	Male	Adult	Good	2018	June	Dry	SP	São Lourenço da Serra	Atlantic forest
RK-253	*Sylvilagus brasiliensis*	Male	Juvenile	Good	2018	May	Dry	SP	São José dos Campos	Atlantic forest
RK-417	*Sylvilagus brasiliensis*	Male	Juvenile	Good	2019	June	Dry	SP	Paraibuna	Atlantic forest

Figure 2. Spatial distribution, and main characteristics of lagomorphs.
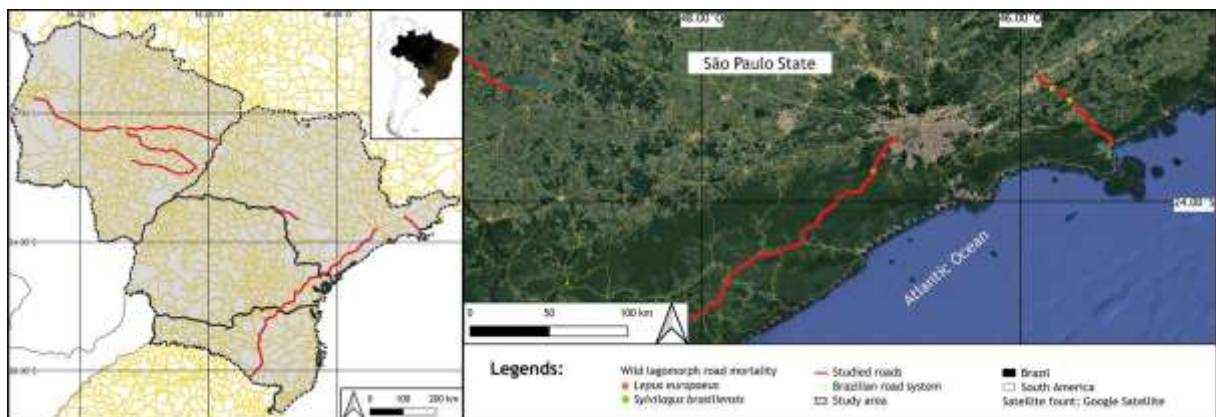
Table 2. Microscopic findings of lagomorphs. European hare (*Lepus europaeus*, n=1), forest rabbit (*Sylvilagus brasiliensis*, n=2).

Species, microscopic findings	N° cases
Lepus europaeus (n=1)	
Carotid	**1**
No significant findings	1
Esophagus	**1**
No significant findings	1
Heart	**1**
No significant findings	1
Jugular	**1**
No significant findings	1
Kidney	**1**
Autolysis	1
Liver	**1**
Autolysis	1
Lungs	**1**
Edema, alveolar	1
Edema, perivascular	1
Hemorrhage, alveolar	1
Hemorrhage, perivascular	1
Small intestine	**1**
Autolysis	1

		Cont.
Tongue		**1**
	Degeneration, ballooning, keratinocytes	1
Trachea		**1**
	No significant findings	1
***Sylvilagus brasiliensis* (n=2)**		
Adrenal glands		**2**
	No significant findings	1
	Autolysis	1
Carotid		**1**
	No significant findings	1
Duodenum		**1**
	Autolysis	1
Esophagus		**2**
	No significant findings	2
Heart		**2**
	No significant findings	1
	Autolysis	1
Jugular		**1**
	No significant findings	1
Kidney		**2**
	No significant findings	1
	Autolysis	2
Large intestine		**1**
	Autolysis	1
Liver		**2**
	No significant findings	1
	Autolysis	2
Lungs		**2**
	Edema, alveolar	2
	Autolysis, moderate	1
	Congestion, capillary beds	1
	Edema, perivascular	1
	Hemorrhage, alveolar	1
	Metazoan, nematode, larvae, adult and L1, alveolar septae and vascular lumen	1
	Pneumonia, granulomatous by lungworm	1
Lymph node		**2**
	No significant findings	1
	Autolysis	1
Pancreas		**1**
	Autolysis	1
Skeletal muscle		**1**
	No significant findings	1
Small intestine		**1**
	Autolysis	1
Spleen		**2**
	Autolysis	2
Stomach		**2**
	Autolysis	2
Thyroid		**1**
	No significant findings	1
Tongue		**2**
	No significant findings	2
Trachea		**2**
	No significant findings	2
Urinary bladder		**1**
	Autolysis	1

APPENDIX VI

General information, gross and histopathological findings in perissodactyls.

Table 1. General information of Lowland tapirs included in this study.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-071	*Tapirus terrestris*	Female	Adult		Good	2017	December	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-074	*Tapirus terrestris*	Male	Adult		Good	2017	December	Rain	MS	Campo Grande	Cerrado
RK-142	*Tapirus terrestris*	Female	Adult		Good	2015	September	Dry	MS	Rio Brilhante	Cerrado
RK-143	*Tapirus terrestris*	Female	Adult		Good	2015	February	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-144	*Tapirus terrestris*	Female	Fetus		Good	2015	February	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-145	*Tapirus terrestris*	Female	Adult		Good	2015	December	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-146	*Tapirus terrestris*	Female	Adult		Good	2016	February	Rain	MS	Terenos	Cerrado
RK-147	*Tapirus terrestris*	Female	Adult		Good	2016	February	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-148	*Tapirus terrestris*	Female	Juvenile		Good	2016	March	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-149	*Tapirus terrestris*	Female	Adult		Good	2016	March	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-150	*Tapirus terrestris*	Female	Juvenile		Good	2016	April	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-151	*Tapirus terrestris*	Male	Juvenile		Good	2016	July	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-152	*Tapirus terrestris*	Male	Adult		Good	2016	May	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-153	*Tapirus terrestris*	Male	Adult		Good	2016	May	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-154	*Tapirus terrestris*	Female	Adult		Good	2016	July	Dry	MS	Campo Grande	Cerrado
RK-155	*Tapirus terrestris*	Female	Adult		Good	2016	July	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-156	*Tapirus terrestris*	Male	Adult		Good	2016	July	Dry	MS	Campo Grande	Cerrado
RK-157	*Tapirus terrestris*	Male	Adult		Good	2016	July	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-158	*Tapirus terrestris*	Male	Juvenile		Good	2016	July	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-159	*Tapirus terrestris*	Male	Adult		Good	2016	July	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-160	*Tapirus terrestris*	Male	Adult		Good	2016	September	Dry	MS	Nova Andradina	Cerrado
RK-161	*Tapirus terrestris*	Male	Adult		Good	2016	October	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-162	*Tapirus terrestris*	Male	Adult		Good	2016	October	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-163	*Tapirus terrestris*	Male	Adult		Good	2016	October	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-164	*Tapirus terrestris*	Male	Adult		Good	2016	November	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-165	*Tapirus terrestris*	Female	Adult		Good	2016	November	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-166	*Tapirus terrestris*	Male	Adult		Good	2016	November	Rain	MS	Dois Irmãos do Buriti	Cerrado
RK-167	*Tapirus terrestris*	Male	Juvenile		Good	2017	February	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-168	*Tapirus terrestris*	Female	Juvenile		Good	2017	April	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-169	*Tapirus terrestris*	Male	Adult		Good	2017	April	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-170	*Tapirus terrestris*	Female	Adult		Good	2017	May	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-171	*Tapirus terrestris*	Male	Adult		Good	2017	May	Dry	MS	Nova Alvorada do Sul	Cerrado
RK-219	*Tapirus terrestris*	Male	Adult		Good	2018	June	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-240	*Tapirus terrestris*	Male	Adult		Good	2018	July	Dry	MS	Campo Grande	Cerrado
RK-244	*Tapirus terrestris*	Male	Adult		Good	2018	July	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-444	*Tapirus terrestris*	Male	Adult	180	Good	2019	December	Rain	MS	Nova Alvorada do Sul	Cerrado

Figure 2. Spatial distribution, and main characteristics of Lowland tapirs included in this study.
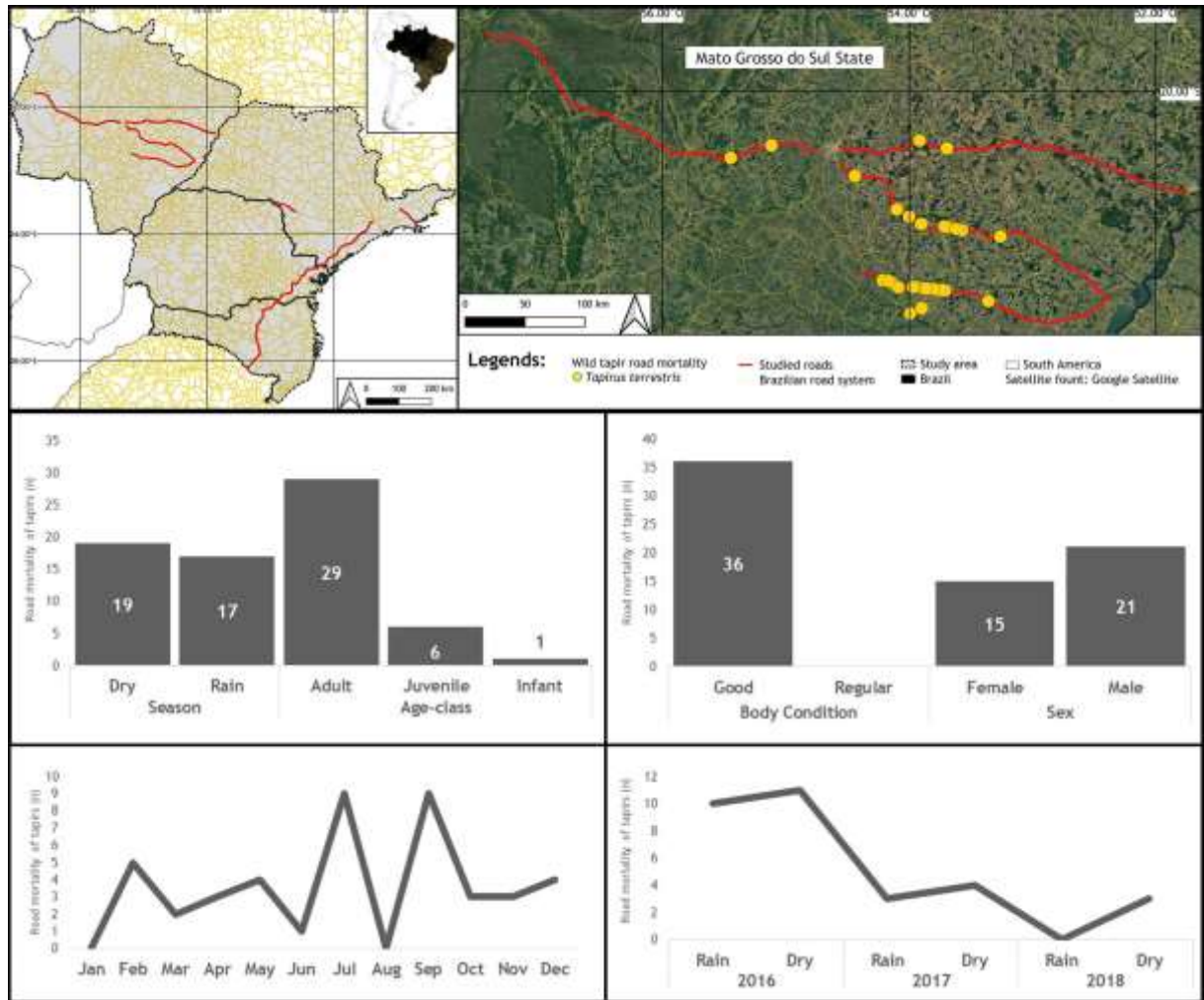
Table 2. Gross findings non-associated with trauma in Lowland tapirs

Species, gross finding	Cases
Tapirus terrestris (n=36)	
Tick infestation	86% (31/36)
Hepatomegaly	28% (10/36)
Yellowish adrenal gland	25% (9/36)
Small intestine, endoparasitic infestation	17% (6/36)
Adrenomegaly	14% (5/36)
Passive hepatic congestion	14% (5/36)
Hepatic degeneration	8% (3/36)
Worn Teeth's	8% (3/36)
Splenomegaly	6% (2/36)
Hepatic abscesses	6% (2/36)

	Cont.
nodular gastritis by nematodes	6% (2/36)
Splenic congestion	6% (2/36)
Vulvar mucous secretion	6% (2/36)
Glossal nodule	3% (1/36)
Hemorrhagic enteritis	3% (1/36)
Hemorrhagic gastritis	3% (1/36)
Hepatic degeneration, suspect	3% (1/36)
Hydroperitoneum, serous	3% (1/36)
Interstitial nephritis	3% (1/36)
Lymph node megaly	3% (1/36)
Parasitic typhlitis	3% (1/36)
Pregnancy	3% (1/36)
Suppurative fasciitis	3% (1/36)
Suppurative pneumonia	3% (1/36)

Figure 2. Main gross findingsin lowland tapirs.

RK-444. *Tapirus terrestris*. Male, adult. Multifocal lacerations.

RK-444. *Tapirus terrestris*. Male, adult. Suppurative fasciitis.

Table 3. Microscopic findings of Lowland tapirs.

Species, microscopic findings	N° cases
***Tapirus terrestris* (n=36)**	
Adrenal glands	**17**
Atrophy, cortex	9
Loss/fibrosis, cortex, fascicular/reticular	9
Fibrosis, cortex	9
Hemorrhage, pericapsular	2
Vacuolar degeneration, cortex, fascicular/reticular	2
Adrenalitis, cortical, mononuclear (lymphocytic)	1
Capsulitis, mononuclear (lymphocytic)	1
No significant findings	6
Autolysis, advanced	1

		Cont.
Brain		**27**
	Hemorrhage	6
	Congestion	1
	Edema, perivascular	1
	Hemorrhage, leptomeningeal	1
	Leukocytic infiltrate, submeningeal, mononuclear (lymphocytic)	1
	Perivascular cuffing, mononuclear (lymphocytic)	1
	No significant findings	16
	Autolysis	2
Carotid		**12**
	Arteritis, mononuclear (lymphocytic, histiocytic), chronic	2
	Serositis, granulocytic (eosinophilic)	1
	Vasa vasorum, subintimal, proliferation-hyperplasia	1
	No significant findings	8
Cecum		**6**
	Colitis, mixed (lymphocytic, eosinophilic)	1
	Metazoan, nematode, larvae, lumen, no inflammation associated	1
	Typhlitis, granulocytic (eosinophilic)	1
	No significant findings	3
	Autolysis	2
Colon		**25**
	Colitis, granulocytic (eosinophilic)	3
	Colitis, mononuclear (lymphocytic, histiocytic), chronic, by ciliates	2
	Colitis, granulocytic (eosinophilic) by ciliates	1
	Colitis, mononuclear (lymphocytic, histiocytic), chronic, by nematode larvae and ciliates	1
	Colitis, mononuclear (lymphocytic, histiocytic), chronic, intra luminal by Strongylida larvae	1
	Colitis, mononuclear (lymphocytic, histiocytic), chronic, lamina propria and submucous, by nematode Strongylida larvae	1
	Leiomyositis, granulocytic (eosinophilic) with fibrosis	1
	No significant findings	17
Diaphragm		**10**
	No significant findings	9
	Autolysis	1
Epididymis		**2**
	Epididymitis, mononuclear (lymphocytic)	1
	Leukocytic infiltrate, interstitial, mononuclear (lymphocytic)	1
Esophagus		**12**
	Esophagitis, granulocytic (eosinophilic)	1
	Esophagitis, mononuclear (lymphocytic)	1
	Esophagitis, subacute	1
	Hemorrhage	1
	No significant findings	9
	Autolysis	1
Gland		**1**
	No significant findings	1
Heart		**33**
	Degeneration, cardiomyocyte	3
	Artery, tunica media, hyperplasia/hypertrophy	2
	Leukocytic infiltrate, mononuclear (lymphocytic)	2
	Arteriosclerosis	1
	Endocarditis, granulocytic (neutrophilic)	1
	Endocarditis, mixed (lymphocytic, eosinophilic)	1
	Fibrosis, myocardium	1
	Leukocytic infiltrate, granulocytic (eosinophilic)	1
	Leukocytic infiltrate, interstitium, granulocytic (eosinophilic)	1

		Cont.
	Myocarditis, mixed (lymphocytic, eosinophilic)	1
	Myocarditis, mononuclear (lymphocytic)	1
	Myocarditis, subendocardial, chronic with fibrosis	1
	Necrosis, cardiomyocyte	1
	No significant findings	21
	Autolysis	3
Kidney		**32**
	Hemorrhage	9
	Degeneration, tubular	6
	Brownish pigment, tubular, cytoplasm	2
	Fibrosis, cortex	2
	Leukocytic infiltrate, perivascular, mononuclear (lymphocytic)	2
	Nephritis, interstitial, mononuclear (lymphocytic)	2
	Proteinosis, tubular	2
	Congestion, cortex	1
	Endoarteritis, mixed (eosinophilic, lymphocytic) with fibrosis	1
	Fibrosis, interlobar	1
	Hemorrhage, medullae	1
	Nephritis, interstitial, mixed (lymphocytic, eosinophilic)	1
	No significant findings	12
	Autolysis	7
Liver		**33**
	Hemorrhage, lobular	5
	Lipofuscinosis	5
	Pericholangitis, granulocytic (eosinophilic)	3
	Binucleation, hepatocyte	2
	Cholangitis/pericholangitis, granulocytic (eosinophilic)	2
	Degeneration, hydropic, hepatocyte	2
	Fibrosis, periportal	2
	Hepatitis, lobular, mononuclear (lymphocytic)	2
	Hepatitis, portal, mixed (eosinophilic, lymphocytic)	2
	Leukocyte infiltration, periportal, mononuclear (lymphocytic)	2
	Necrosis/apoptosis, hepatocyte, single cell	2
	Pericholangitis, mixed (eosinophilic, lymphocytic)	2
	Arteriole, tunica media, hyperplasia	1
	Cholangitis, granulocytic (eosinophilic)	1
	Cholangitis, mixed (lymphocytic, eosinophilic)	1
	Congestion, portal	1
	Congestion, sinusoidal	1
	Fibrosis, portal, P-P bridging	1
	Hematomieloid nodule	1
	Hemorrhage, portal	1
	Hepatic abscess with fibrosis, mineralization, and bacterial colonies.	1
	Hepatitis, interface, mononuclear (lymphocytic, histiocytic)	1
	Hepatitis, portal, mononuclear (lymphocytic)	1
	Leukocyte infiltration, portal, granulocytic (eosinophilic)	1
	Leukocyte infiltration, portal, mononuclear (lymphocytic)	1
	Leukocyte infiltration, sinusoid-portal, mononuclear (lymphocytic)	1
	Leukocyte infiltration, sinusoids, mixed (neutrophilic, lymphocytic)	1
	Metazoan, nematode, microfilaries, intravascular, no inflammation	1
	Pericholangitis, mixed (lymphocytic, plasmacytic, eosinophilic)	1
	Tubular cyst	1
	Vascular, hyperplasia	1
	No significant findings	6
	Autolysis	6
Lungs		**35**
	Hemorrhage, alveolar	19

Cont.

Anthracosis	11
Hyperinsuflation, alveolar	11
Pneumonia, interstitial, granulocytic (eosinophilic)	9
Pneumonia, interstitial, mononuclear (lymphocytic)	9
Artery, tunica media, hypertrophy	6
Edema, alveolar	6
Congestion, capillary beds	5
Atelectasis	4
Fibrosis	3
Agonic aspiration, alveoli	2
Autolysis, advanced	2
Bronchiolitis, granulocytic (eosinophilic)	2
Fibrosis, interlobar	2
Rupture, alveolar	2
Agonic aspiration, alveoli, keratin squames and meconium plugs	1
Anthracosis, peribronchial	1
BALT hyperplasia	1
Bronchitis, granulocytic (eosinophylic)	1
Bronchitis, mononuclear (lymphocytic)	1
Bronchopneumonia, abscedative with bacterial emboli	1
Bronchopneumonia, mixed (eosinophilic, histiocytic)	1
Bullae, subpleural	1
Edema, interlobar	1
Edema, perivascular	1
Edema, subpleural	1
Hemosiderosis	1
Histiocytosis, alveolar	1
Leukocyte infiltration, perivascular, mixed (lymphocytic, plasmacytic, eosinophilic)	1
Lymphangiectasis, subpleural	1
Pleuritis, granulocytic (eosinophilic)	1
Pleuritis, granulomatous by lungworm	1
Pleuritis, mononuclear (lymphocytic, histiocytic), chronic	1
Pneumocyte, hyperplasia	1
Pneumonia, bronchointerstitial, mononuclear (lymphocytic, histiocytic) with MNGC, potential aspiration	1
Pneumonia, interstitial, mixed (eosinophylic, lymphocytic), lobar, septal	1
Lymph node	**30**
Hemorrhage, sinusal	8
Paracortical lymphoid reactive hyperplasia	6
Lymphadenitis, granulomatous	3
Hemorrhage, perinodal adipose tissue	1
Hemosiderosis	1
Histiocytosis, sinusal	1
Lymphadenitis, granulocytic (eosinophilic)	1
Lymphadenitis, granulocytic (neutrophilic)	1
Lymphadenitis, granulomatous with presume or equivocal nematode larva	1
Lymphoid depletion	1
Plasmocitosis	1
Sinus, erythrocytosis	1
No significant findings	7
Autolysis	1
Mammary gland	**2**
Mastitis, mononuclear (lymphocytic, plasmacytic)	2
Pancreas	**8**
No significant findings	6
Autolysis	2

Cont.

Salivary gland	**1**
No significant findings	1
Skeletal muscle	**11**
Degeneration, myofiber	2
Degeneration/necrosis, myofiber	1
Necrosis, myofiber	1
No significant findings	6
Autolysis	2
Skin	**1**
No significant findings	1
Small intestine	**31**
Enteritis, granulocytic (eosinophylic)	8
Enteritis, granulocytic (eosinophylic) by ciliates	3
Hemorrhage, serosa	2
Leukocyte infiltration, granulocytic (eosinophilic)	2
Arteritis, chronic	1
Enteritis, granulocytic (eosinophylic) by nematodes	1
Enteritis, mixed (lymphocytic, eosinophilic) by ciliates	1
Enteritis, mixed (lymphocytic, eosinophilic) by ciliates and nematodes	1
Enteritis, mononuclear (Lymphocytic)	1
Leukocyte infiltration, mononuclear (lymphocytic)	1
Metazoan, nematode, larvae, adult, lumen	1
Serositis, granulocytic (eosinophilic)	1
No significant findings	10
Autolysis	3
Spleen	**31**
Hemorrhage, red pulp	17
Congestion	9
Hemosiderosis	5
White pulp depletion	4
Extramedular hematopoiesis	3
Histiocytosis, red pulp	1
Laceration, parenchyma	1
Leukocytic infiltrate, red pulp, granulocytic (eosinophilic)	1
Splenitis, granulocytic (eosinophilic)	1
Splenitis, granulomatous	1
No significant findings	6
Autolysis	4
Stomach	**29**
Gastritis, mononuclear (lymphocytic)	2
Gastritis, proliferative and fibrosing, eosinophilic with intralesional nematodes	2
Gastritis, granulocytic (neutrophilic)	1
Gastritis, mixed (eosinophylic, lymphocytic)	1
Granuloma, with mineralization	1
Hemorrhage, serosa	1
Hemorrhage, submucosa	1
Leiomyositis, granulocytic (eosinophilic)	1
Leiomyositis, mononuclear (lymphocytic)	1
Leukocyte infiltrate, granulocytic (eosinophilic)	1
Vasculitis, granulocytic (eosinophylic)	1
No significant findings	17
Autolysis	2
Testicle	**6**
Orchitis, mononuclear (lymphocytic, plasmacytic)	2
Hemorrhage	1
No significant findings	3

	Cont.
TGI	**1**
Arthropod, undetermined, lumen	1
Tongue	**22**
Endoarteritis, proliferative and fibrosing, chronic (lymphocytic, histiocytic) with stenosis	2
Glossitis, granulomatous by foreign body	2
Glossitis, superficial, mononuclear (histiocytic, lymphocytic)	2
Hemorrhage, muscular layer	2
Artery, arteriosclerosis, chronic	1
Artery, subintimal, hyperplasia	1
Epithelium, hyperplasia	1
Fibrosis	1
Glossitis, granulocytic (eosinophylic)	1
Glossitis, granulomatous, nodular	1
Glossitis, internal, mixed (neutrophilic, histiocytic, lymphocytic)	1
Glossitis, internal, mononuclear (histiocytic)	1
Glossitis, internal, mononuclear (histiocytic, lymphocytic)	1
Glossitis, mixed (eosinophylic, lymphocytic, plasmacytic)	1
Glossitis, mononuclear (histiocytic, lymphocytic)	1
Glossitis, mononuclear (lymphocytic)	1
Glossitis, superficial, granulocytic (neutrophilic)	1
Glossitis, superficial, mixed (eosinophilic, lymphocytic)	1
No significant findings	11
Trachea	**25**
Tracheitis, mixed (eosinophilic, lymphocytic)	3
Tracheitis, granulocytic (eosinophilic)	2
Tracheitis, mononuclear (lymphocytic)	2
Tracheitis, mononuclear (lymphocytic, plasmacytic)	2
Hemorrhage, mucosa	1
Tracheitis, mixed (eosinophilic, lymphocytic, plasmacytic) with intravascular bacteria	1
Tracheitis, mononuclear (lymphocytic, histiocytic)	1
No significant findings	12
Autolysis, advanced	1
Urethra	**1**
Urethritis, perivascular, mononuclear (lymphocytic, plasmacytic)	1
Urinary bladder	**12**
Hemorrhage, perivascular adipose tissue	1
No significant findings	11
Uterus	**7**
No significant findings	7

APPENDIX VII

General information, gross and histopathological findings in anteaters and sloths.

Table 1. General information of the sloths and anteaters.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-011	*Bradypus variegatus*	Male	Juvenile	3,9	Regular	2016	March	Rain	SP	Juquitiba	Atlantic forest
RK-018	*Bradypus variegatus*	Male	Adult	4,7	Regular	2015	April	Dry	SP	Miracatu	Atlantic forest
RK-209	*Bradypus variegatus*	Male	Juvenile	4,2	Good	2018	January	Rain	SP	São Lourenço da Serra	Atlantic forest
RK-210	*Bradypus variegatus*	Male	Juvenile	4	Regular	2018	April	Rain	SP	Registro	Atlantic forest
RK-429	*Bradypus variegatus*	Female	Adult	4,9	Good	2019	October	Rain	SP	Jambeiro	Atlantic forest
RK-092	*Myrmecophaga tridactyla*	Male	Adult		Good	2017	January	Rain	MS	Anastácio	Cerrado
RK-093	*Myrmecophaga tridactyla*	Male	Adult		Good	2017	January	Rain	MS	Anastácio	Cerrado
RK-094	*Myrmecophaga tridactyla*	Male	Adult		Good	2017	February	Rain	MS	Dois Irmãos do Buriti	Cerrado
RK-095	*Myrmecophaga tridactyla*	Unknown	Juvenile	19,2	Good	2017	February	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-096	*Myrmecophaga tridactyla*	Female	Adult	24,0	Good	2017	February	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-097	*Myrmecophaga tridactyla*	Male	Adult		Good	2017	February	Rain	MS	Miranda	Pantanal
RK-098	*Myrmecophaga tridactyla*	Male	Adult	31,0	Good	2017	March	Rain	MS	Anastácio	Cerrado
RK-099	*Myrmecophaga tridactyla*	Male	Adult		Good	2017	March	Rain	MS	Campo Grande	Cerrado
RK-101	*Myrmecophaga tridactyla*	Female	Adult		Good	2017	March	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-103	*Myrmecophaga tridactyla*	Female	Adult	24,4	Good	2017	March	Rain	MS	Santa Rita do Pardo	Cerrado
RK-105	*Myrmecophaga tridactyla*	Male	Adult	37,0	Good	2017	March	Rain	MS	Campo Grande	Cerrado
RK-106	*Myrmecophaga tridactyla*	Female	Adult	31,0	Good	2017	April	Dry	MS	Terenos	Cerrado
RK-109	*Myrmecophaga tridactyla*	Male	Adult		Good	2017	May	Dry	MS	Dois Irmãos do Buriti	Cerrado
RK-113	*Myrmecophaga tridactyla*	Female	Adult	31,1	Good	2017	May	Dry	MS	Anastácio	Cerrado
RK-115	*Myrmecophaga tridactyla*	Male	Infant	4,6	Good	2017	June	Dry	MS	Campo Grande	Cerrado
RK-116	*Myrmecophaga tridactyla*	Female	Adult	35,0	Good	2017	June	Dry	MS	Campo Grande	Cerrado
RK-117	*Myrmecophaga tridactyla*	Male	Adult	27,0	Good	2017	June	Dry	MS	Santa Rita do Pardo	Cerrado
RK-118	*Myrmecophaga tridactyla*	Female	Adult		Good	2017	July	Dry	MS	Miranda	Pantanal
RK-119	*Myrmecophaga tridactyla*	Female	Infant	1,1	Good	2017	July	Dry	MS	Unknown	Cerrado
RK-120	*Myrmecophaga tridactyla*	Male	Adult	23,3	Good	2017	July	Dry	MS	Campo Grande	Cerrado
RK-121	*Myrmecophaga tridactyla*	Male	Adult	38,6	Good	2017	July	Dry	MS	Terenos	Cerrado
RK-122	*Myrmecophaga tridactyla*	Female	Infant	1,2	Poor	2017	July	Dry	MS	Unknown	Cerrado
RK-123	*Myrmecophaga tridactyla*	Female	Adult	27,7	Poor	2017	July	Dry	MS	Unknown	Cerrado
RK-125	*Myrmecophaga tridactyla*	Male	Adult	42,0	Good	2017	August	Dry	MS	Terenos	Cerrado
RK-126	*Myrmecophaga tridactyla*	Male	Infant	5,8	Poor	2017	August	Dry	MS	Campo Grande	Cerrado
RK-127	*Myrmecophaga tridactyla*	Male	Adult	28,0	Good	2017	August	Dry	MS	Ribas do Rio Pardo	Cerrado

RK-128	*Myrmecophaga tridactyla*	Male	Adult	22,8	Poor	2017	August	Dry	MS	Unknown	Cerrado
RK-130	*Myrmecophaga tridactyla*	Female	Adult	25,7	Good	2017	September	Dry	MS	Dois Irmãos do Buriti	Cerrado
RK-131	*Myrmecophaga tridactyla*	Male	Adult	35,5	Good	2017	September	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-132	*Myrmecophaga tridactyla*	Female	Adult	33,0	Good	2017	September	Dry	MS	Miranda	Cerrado
RK-134	*Myrmecophaga tridactyla*	Male	Adult	27,5	Regular	2017	October	Rain	MS	Campo Grande	Cerrado
RK-135	*Myrmecophaga tridactyla*	Male	Adult	28,5	Good	2017	October	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-184	*Myrmecophaga tridactyla*	Male	Adult	29,8	Regular	2018	January	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-187	*Myrmecophaga tridactyla*	Male	Adult	27,3	Good	2018	February	Rain	MS	Campo Grande	Cerrado
RK-190	*Myrmecophaga tridactyla*	Male	Adult	30,0	Good	2018	February	Rain	MS	Campo Grande	Cerrado
RK-191	*Myrmecophaga tridactyla*	Male	Adult	28,3	Good	2018	February	Rain	MS	Bonito	Cerrado
RK-192	*Myrmecophaga tridactyla*	Male	Adult	29,5	Good	2018	March	Rain	MS	Terenos	Cerrado
RK-193	*Myrmecophaga tridactyla*	Female	Adult	40,0	Regular	2018	March	Rain	MS	Campo Grande	Cerrado
RK-194	*Myrmecophaga tridactyla*	Male	Adult	42,7	Good	2018	March	Rain	MS	Miranda	Pantanal
RK-195	*Myrmecophaga tridactyla*	Male	Juvenile	21,7	Regular	2018	April	Dry	MS	Campo Grande	Cerrado
RK-196	*Myrmecophaga tridactyla*	Male	Adult	28,9	Good	2018	April	Dry	MS	Unknown	Cerrado
RK-225	*Myrmecophaga tridactyla*	Female	Adult	26	Good	2018	April	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-226	*Myrmecophaga tridactyla*	Female	Juvenile	17	Good	2018	April	Dry	MS	Dois Irmãos do Buriti	Cerrado
RK-227	*Myrmecophaga tridactyla*	Female	Adult	31,8	Good	2018	May	Dry	MS	Santa Rita do Pardo	Cerrado
RK-228	*Myrmecophaga tridactyla*	Female	Adult	30	Good	2018	May	Dry	MS	Ribas do Rio Pardo	Cerrado
RK-230	*Myrmecophaga tridactyla*	Male	Adult	26	Good	2018	June	Dry	MS	Anastácio	Cerrado
RK-232	*Myrmecophaga tridactyla*	Male	Adult	35,3	Good	2018	June	Dry	MS	Aquidauana	Pantanal
RK-233	*Myrmecophaga tridactyla*	Male	Adult	27,6	Good	2018	June	Dry	MS	Dois Irmãos do Buriti	Cerrado
RK-234	*Myrmecophaga tridactyla*	Male	Adult	37,8	Good	2018	April	Dry	MS	Santa Rita do Pardo	Cerrado
RK-241	*Myrmecophaga tridactyla*	Male	Adult	29,9	Good	2018	July	Dry	MS	Campo Grande	Cerrado
RK-266	*Myrmecophaga tridactyla*	Female	Adult	34	Good	2018	September	Dry	MS	Santa Rita do Pardo	Cerrado
RK-267	*Myrmecophaga tridactyla*	Male	Adult	28,6	Good	2018	August	Dry	MS	Nova Andradina	Cerrado
RK-288	*Myrmecophaga tridactyla*	Male	Adult	35,8	Good	2018	October	Rain	MS	Aquidauana	Pantanal
RK-293	*Myrmecophaga tridactyla*	Male	Juvenile		Good	2018	November	Rain	MS	Santa Rita do Pardo	Cerrado
RK-319	*Myrmecophaga tridactyla*	Female	Adult	31,6	Good	2019	January	Rain	MS	Terenos	Cerrado
RK-322	*Myrmecophaga tridactyla*	Female	Juvenile	22,5	Good	2019	February	Rain	MS	Campo Grande	Cerrado
RK-401	*Myrmecophaga tridactyla*	Female	Adult	30,2	Good	2019	May	Dry	MS	Miranda	Pantanal
RK-406	*Myrmecophaga tridactyla*	Female	Juvenile		Good	2019	July	Dry	MS	Dois Irmãos do Buriti	Cerrado
RK-435	*Myrmecophaga tridactyla*	Male	Adult		Good	2019	November	Rain	MS	Anastácio	Cerrado
RK-440	*Myrmecophaga tridactyla*	Male	Juvenile	18	Good	2019	December	Rain	MS	Anastácio	Cerrado
RK-452	*Myrmecophaga tridactyla*	Male	Adult		Good	2018	September	Dry	MS	Santa Rita do Pardo	Cerrado
RK-453	*Myrmecophaga tridactyla*	Female	Adult		Good	2019	April	Dry	MS	Campo grande	Cerrado
RK-003	*Tamandua tetradactyla*	Male	Juvenile	3,1	Good	2016	December	Rain	SP	Miracatu	Atlantic forest
RK-005	*Tamandua tetradactyla*	Female	Juvenile	3,7	Regular	2016	December	Rain	SP	Miracatu	Atlantic forest

RK-006	*Tamandua tetradactyla*	Female	Juvenile	3,9	Regular	2016	October	Rain	SP	Miracatu	Atlantic forest
RK-013	*Tamandua tetradactyla*	Female	Adult	4,5	Good	2014	December	Rain	SP	Registro	Atlantic forest
RK-048	*Tamandua tetradactyla*	Female	Adult	6,7	Good	2017	May	Dry	SP	Paraibuna	Atlantic forest
RK-053	*Tamandua tetradactyla*	Male	Juvenile	3,7	Poor	2014	August	Dry	SP	Miracatu	Atlantic forest
RK-081	*Tamandua tetradactyla*	Female	Adult	5,8	Regular	2017	October	Rain	SP	Paraibuna	Atlantic forest
RK-107	*Tamandua tetradactyla*	Male	Adult	5,8	Good	2017	April	Dry	MS	Anastácio	Cerrado
RK-110	*Tamandua tetradactyla*	Female	Adult	4,4	Good	2017	May	Dry	MS	Miranda	Pantanal
RK-112	*Tamandua tetradactyla*	Male	Adult	8,0	Good	2017	May	Dry	MS	Campo Grande	Cerrado
RK-124	*Tamandua tetradactyla*	Female	Adult	4,7	Good	2017	July	Dry	MS	Santa Rita do Pardo	Cerrado
RK-177	*Tamandua tetradactyla*	Male	Adult	4,8	Good	2017	December	Rain	SP	Caraguatatuba	Atlantic forest
RK-185	*Tamandua tetradactyla*	Female	Adult	5,8	Good	2018	February	Rain	MS	Dois Irmãos do Buriti	Cerrado
RK-189	*Tamandua tetradactyla*	Female	Adult	5,0	Good	2018	February	Rain	MS	Anastácio	Cerrado
RK-197	*Tamandua tetradactyla*	Male	Juvenile	2,7	Good	2018	April	Dry	MS	Unknown	Cerrado
RK-231	*Tamandua tetradactyla*	Male	Adult	6,8	Good	2018	June	Dry	MS	Anastácio	Cerrado
RK-237	*Tamandua tetradactyla*	Male	Adult		Good	2018	July	Dry	MS	Dois Irmãos do Buriti	Cerrado
RK-238	*Tamandua tetradactyla*	Male	Adult	5,4	Good	2018	July	Dry	MS	Terenos	Cerrado
RK-248	*Tamandua tetradactyla*	Male	Adult	7,2	Good	2018	July	Dry	MS	Campo Grande	Cerrado
RK-251	*Tamandua tetradactyla*	Female	Adult		Good	2018	August	Dry	SP	Ipaussu	Cerrado
RK-256	*Tamandua tetradactyla*	Female	Juvenile	6,3	Good	2018	October	Rain	SP	São José dos Campos	Atlantic forest
RK-283	*Tamandua tetradactyla*	Male	Adult	7,8	Good	2018	December	Rain	SP	Ipaussu	Cerrado
RK-289	*Tamandua tetradactyla*	Female	Adult	5,35	Good	2018	October	Rain	MS	Nova Alvorada do Sul	Cerrado
RK-290	*Tamandua tetradactyla*	Male	Adult	5,7	Good	2018	November	Rain	MS	Terenos	Cerrado
RK-291	*Tamandua tetradactyla*	Male	Adult		Good	2018	November	Rain	MS	Ribas do Rio Pardo	Cerrado
RK-301	*Tamandua tetradactyla*	Female	Adult	5,2	Good	2019	February	Rain	SP	Chavantes	Cerrado
RK-302	*Tamandua tetradactyla*	Female	Adult	6	Good	2018	December	Rain	SP	Caraguatatuba	Atlantic forest
RK-321	*Tamandua tetradactyla*	Female	Adult	5,4	Good	2019	March	Rain	MS	Anastácio	Cerrado
RK-328	*Tamandua tetradactyla*	Male	Adult	5,8	Good	2018	November	Rain	SC	São Francisco do Sul	Atlantic forest
RK-343	*Tamandua tetradactyla*	Male	Adult	8,28	Good	2015	November	Rain	SC	São Francisco do Sul	Atlantic forest
RK-347	*Tamandua tetradactyla*	Female	Adult	6,26	Good	2015	December	Rain	SC	São Francisco do Sul	Atlantic forest
RK-350	*Tamandua tetradactyla*	Male	Adult	6,03	Good	2016	January	Rain	SC	São Francisco do Sul	Atlantic forest
RK-355	*Tamandua tetradactyla*	Male	Adult	6,35	Good	2018	January	Rain	SC	Itapoá	Atlantic forest
RK-372	*Tamandua tetradactyla*	Female	Adult	4,88	Good	2017	July	Dry	SC	São Francisco do Sul	Atlantic forest
RK-374	*Tamandua tetradactyla*	Female	Adult	5,05	Good	2019	April	Dry	SC	Itaiópolis	Atlantic forest
RK-394	*Tamandua tetradactyla*	Female	Adult	5,29	Good	2014	June	Dry	SC	Joinville	Atlantic forest
RK-399	*Tamandua tetradactyla*	Female	Adult	4,4	Good	2019	July	Dry	SP	Piraju	Cerrado
RK-415	*Tamandua tetradactyla*	Male	Adult	5,2	Good	2019	June	Dry	SP	Caraguatatuba	Atlantic forest
RK-433	*Tamandua tetradactyla*	Male	Adult	7,6	Good	2019	November	Rain	SP	Paraibuna	Atlantic forest
RK-434	*Tamandua tetradactyla*	Male	Adult		Good	2019	November	Rain	MS	Terenos	Cerrado

Figure 1. Spatial distribution, and main characteristics of collared anteaters.
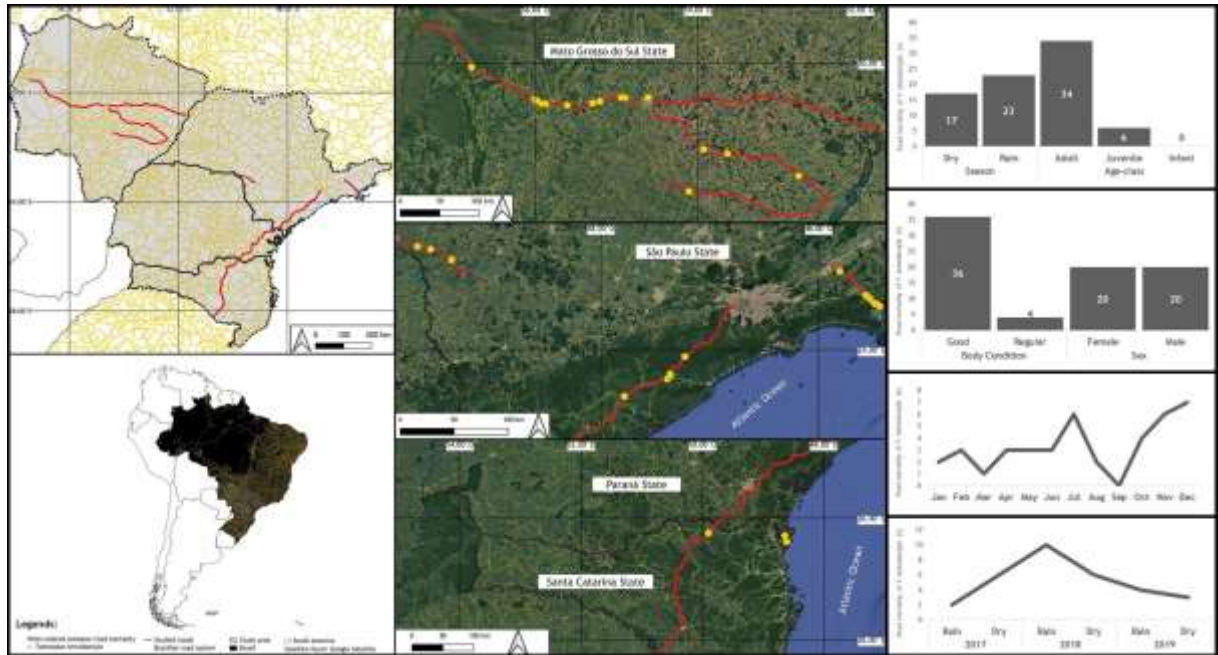
Figure 2. Spatial distribution, and main characteristics of sloths.
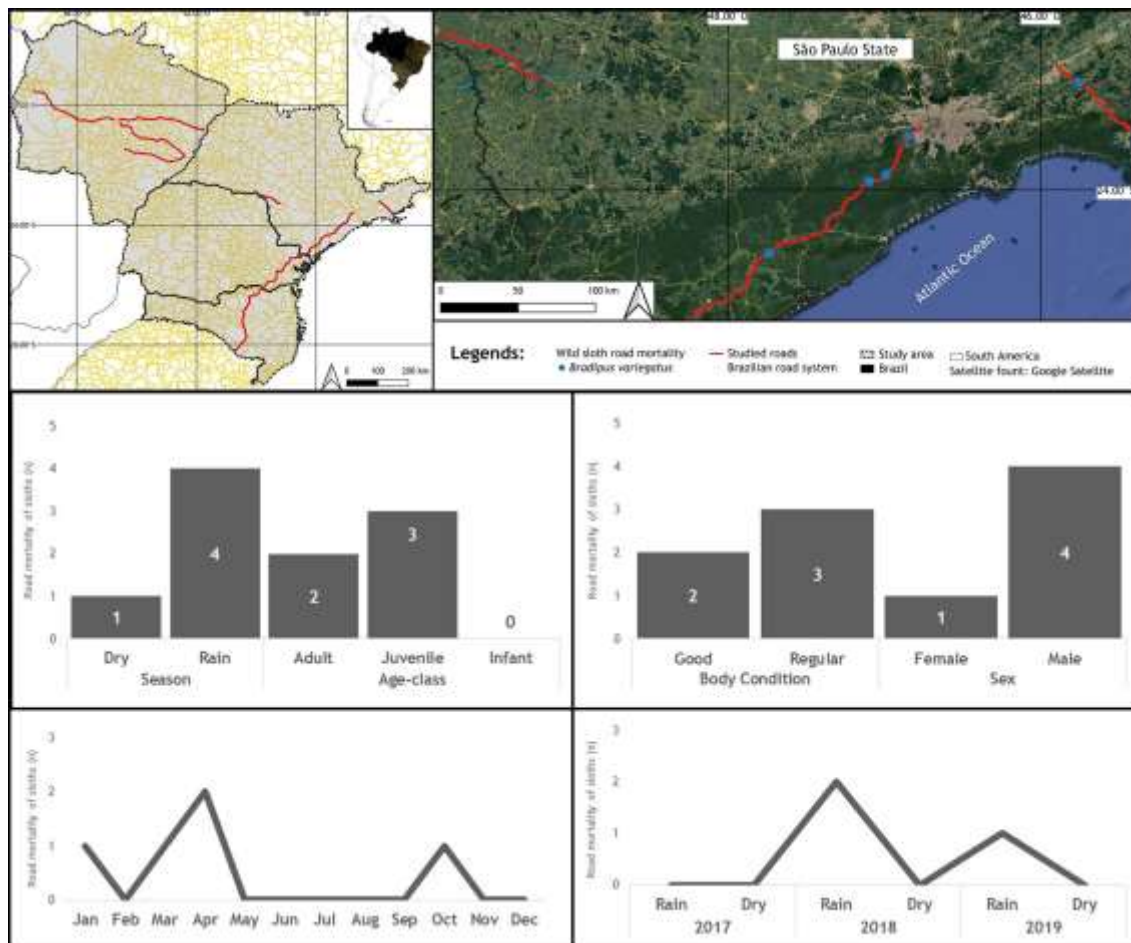
Figure 3. Spatial distribution, and main characteristics of giant anteaters.
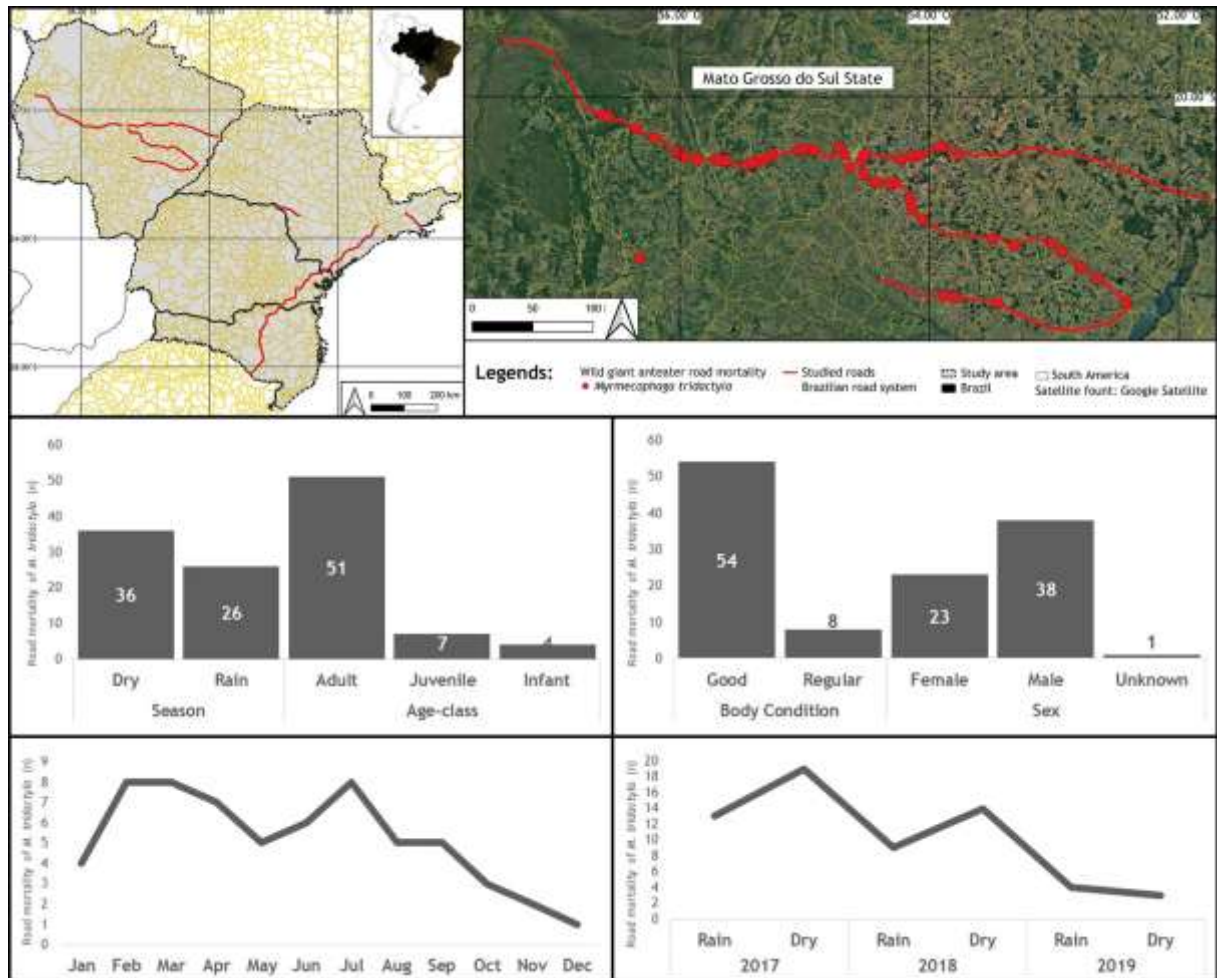
Table 2. Gross findings non-associated with trauma in sloths and anteaters.

Species, gross finding	Cases
Bradypus variegatus **(n=5)**	
Tick infestation	60% (3/5)
Consolidated fracture	20% (1/5)
Splenic white pulp hyperplasia	20% (1/5)
Small intestine, endoparasitic infestation	20% (1/5)
Myrmecophaga tridactyla **(n=61)**	
Stomach, endoparasitic infestation	54% (33/61)
Tick infestation	54% (33/61)
Small intestine, endoparasitic infestation	46% (28/61)
Brain congestion	20% (12/61)
Mesenteric lymph node megaly	13% (8/61)
Splenomegaly	7% (4/61)
Gallbladder, distended	7% (4/61)
Passive hepatic congestion	5% (3/61)
Pregnancy	3% (2/61)
Hepatic degeneration	3% (2/61)
Dermal pustule	2% (1/61)
Disgusted nail	2% (1/61)

	Cont.
Enteritis by acanthocephala	2% (1/61)
Splenic white pulp hyperplasia	2% (1/61)
Gastric ulcer	2% (1/61)
Hemorrhagic cystitis	2% (1/61)
Hepatic congestion	2% (1/61)
Lactancy	2% (1/61)
Lymph node hemorrhage	2% (1/61)
Ocular seromucous secretion	2% (1/61)
Parasitic enteritis	2% (1/61)
Splenic cyst	2% (1/61)
Tamandua tetradactyla (n=40)	
Tick infestation	55% (22/40)
Lymph node megaly	30% (12/40)
Small intestine, endoparasitic infestation	18% (7/40)
Ocular seromucous secretion	8% (3/40)
Parasitic enteritis	8% (3/40)
Splenic white pulp hyperplasia	5% (2/40)
Large intestine, endoparasitic infestation	5% (2/40)
Splenomegaly	3% (1/40)
Gallbladder, distended	3% (1/40)
Gastritis	3% (1/40)
Hepatic degeneration	3% (1/40)
Hepatitis, suspect	3% (1/40)
Lymph node hemorrhage	3% (1/40)
Passive hepatic congestion	3% (1/40)
Predation	3% (1/40)
Pregnancy	3% (1/40)
Regular body condition	3% (1/40)
Skin crust	3% (1/40)
Stomach, endoparasitic infestation	3% (1/40)

Figure 4. Main gross findings in anteaters and slots.

RK-048. *Tamandua tetradactyla*. Female, adult. Tick, Family: *Ixodidae*, genus: *Amblyomma*.

RK-119. *Myrmecophaga tridactyla*. Female, infant. Tick, Family: *Ixodidae*, genus: *Amblyomma*.

RK-048. *Tamandua tetradactyla*. Female, adult. Intestine. Metazoan parasite. Acantocephala.

RK-135. *Myrmecophaga tridactyla*. Male, adult. Stomach. Metazoan parasite. Nematode.

RK-081. *Tamandua tetradactyla*. Female, adult. Pregnancy.

RK-193. *Myrmecophaga tridactyla*. Female, adult. Pregnancy.

Table 3. Microscopic findings of sloths and anteaters. Brown-throated Three-toed Sloth (*Bradypus variegatus*, n=5), giant anteater (*Myrmecophaga tridactyla*, n=61), Collared anteater (*Tamandua tetradactyla*, n=41).

Species, microscopic findings	N° cases
***Bradypus variegatus* (n=5)**	
Brain	**2**
Autolysis	2
Esophagus	**4**
No significant findings	4
Heart	**5**
No significant findings	5
Kidney	**5**
No significant findings	1
Autolysis	4
Liver	**5**
Hepatitis, lobular, granulocytic (neutrophilic)	1
Hepatitis, lobular, mixed (neutrophilic, histiocytic)	1
Hepatitis, portal, mononuclear (lymphocytic, histiocytic)	1
No significant findings	1
Autolysis	1

Cont.

Lungs	**5**
Edema, perivascular	5
Edema, alveolar	4
Edema, subpleural	4
Hyperinsuflation, alveolar	2
Agonic aspiration, alveoli	1
Anthracosis	1
Edema, interlobar	1
Extramedular thrombopoiesis	1
Hemosiderosis	1
Salivary gland	**4**
No significant findings	3
Autolysis	1
Skeletal muscle	**1**
No significant findings	1
Skin	**1**
No significant findings	1
Small intestine	**4**
No significant findings	3
Autolysis	1
Spleen	**2**
Extramedular hematopoiesis	1
White pulp hyperplasia	1
No significant findings	1
Stomach	**3**
No significant findings	3
Testicle	**3**
Sexual maturity	2
Autolysis	1
Tongue	**2**
Glossitis, superficial, mixed (eosinophilic, lymphocytic)	1
No significant findings	1
Trachea	**5**
Hemorrhage, serosa	1
No significant findings	4
Ureter	**1**
No significant findings	1
Urinary bladder	**2**
No significant findings	1
Autolysis	1
Uterus	**1**
No significant findings	1

***Myrmecophaga tridactyla* (n=61)**	
Adrenal glands	**34**
Hemorrhage, pericapsular	8
Hemorrhage, cortical, fascicular	7
Hemorrhage, cortical, fascicular/reticular	7
Loss/fibrosis, cortex, fascicular/reticular	7
Cortical hemorrhage	3
Hemorrhage, cortical, reticular	3
Vacuolar degeneration, cortex, glomerular	3
Fibrosis, cortex	2
Hemorrhage, cortical	2
Vacuolar degeneration, cortex, fascicular/reticular	2
Atrophy, cortex	1
Congestion, cortex	1

	Congestion, cortical, fascicular/reticular	1
	Cortical glomerular, fascicular hemorrhage	1
	Necrosis, cortical layer (fascicular, reticular)	1
	Vacuolar degeneration, cortex, fascicular	1
	No significant findings	5
	Autolysis	1
Brain		**35**
	Hemorrhage, leptomeningeal/neuroparenchyma	5
	Hemorrhage, neuroparenchyma	4
	Hemorrhage, leptomeningeal	4
	Satellitosis	3
	Edema, perivascular	2
	Congestion, leptomeningeal	1
	Encephalitis	1
	Gliosis	1
	Perivascular cuffing, mononuclear (lymphocytic, histiocytic)	1
	No significant findings	18
	Autolysis	2
Carotid		**7**
	No significant findings	7
Cerebellum		**23**
	Hemorrhage, leptomeningeal	3
	Hemorrhage, leptomeningeal/neuroparenchyma	3
	Congestion	2
	Purkinje cells hipereosinophilia	1
	No significant findings	14
Diaphragm		**17**
	Degeneration, myofiber	3
	Myositis, granulocytic (neutrophilic)	2
	Protozoa, sarcocystid, Intrasarcolemal, no inflammation	2
	Necrosis, myofiber	1
	No significant findings	10
	Autolysis	1
Esophagus		**42**
	Hemorrhage, serosa	9
	Esophagitis, mononuclear (lymphocytic)	2
	Hemorrhage, submucosa	2
	Esophagitis, ulcerative, granulocytic (neutrophilic)	1
	No significant findings	29
Gland		**7**
	No significant findings	7
Heart		**50**
	Degeneration, cardiomyocyte	3
	Hemorrhage, myocardial	3
	Cardiomyocyte, perinuclear vacuolation	2
	Myocarditis, mononuclear (lymphocytic)	2
	Myocarditis, mononuclear (lymphocytic, histiocytic)	2
	Hemorrhage	1
	Leukocytic infiltrate, perivascular, mononuclear (lymphocytic)	1
	Myocarditis, granulocytic (neutrophilic)	1
	Necrosis, cardiomyocyte	1
	No significant findings	35
	Autolysis	3
Jugular		**1**
	No significant findings	1
Kidney		**56**
	Proteinosis, tubular	10

Cont.

Nephritis, interstitial, mononuclear (lymphocytic)	6
Degeneration, tubular	4
Proteinosis, tubular/glomerular	4
Hemorrhage, pericapsular	3
MALT hyperplasia, medullae	3
Congestion, cortex, glomerular	2
Congestion, corticomedular	2
Hemorrhage, cortex	2
Hemorrhage, tubulointerstitial	2
Brownish pigment, tubular, cytoplasm	1
Fibrosis, cortex	1
Glomerulonephritis, membranous	1
Hemorrhage, corticomedular	1
Leukocytic infiltrate, perivascular, mononuclear (lymphocytic)	1
Mineralization, tubular, medullae	1
Necrosis, tubular	1
Necrosis, tubular, acute	1
Proteinosis, glomerular	1
No significant findings	16
Autolysis	17
Large intestine	**7**
No significant findings	5
Autolysis	2
Liver	**54**
Congestion, sinusoidal	13
Hemorrhage, lobular	9
Hepatitis, lobular, mononuclear (lymphocytic, histiocytic)	7
Degeneration, hydropic, hepatocyte	5
Degeneration, steatosis, macrogoticular, hepatocyte	5
Hepatitis, lobular, mixed (neutrophilic, histiocytic)	3
Leukocyte infiltration, portal, mononuclear (lymphocytic, histiocytic)	3
Necrosis/apoptosis, hepatocyte, single cell	3
Binucleation, hepatocyte	2
Brownish pigment, hepatocyte, cytoplasm	2
Ductular reaction, bile duct	2
Leukocyte infiltration, periductular, mononuclear (lymphocytic)	2
Cholangitis, mononuclear (lymphocytic, histiocytic)	1
Degeneration, steatosis, microgoticular, hepatocyte	1
Detrabeculation, hepatocyte	1
Hemorrhage, periportal	1
Hemorrhage, portal	1
Hepatitis, lobular, necrotizing, mixed (lymphocytic, neutrophilic)	1
Hepatitis, portal, mixed (eosinophylic, lymphocytic)	1
Hepatocytes, intranuclear inclusion	1
Leukocyte infiltration, perivascular/periductular, mixed (lymphocytic, eosinophilic)	1
Pericholangitis, mononuclear (lymphocytic)	1
No significant findings	14
Autolysis	11
Lungs	**56**
Hemorrhage, alveolar	49
Edema, alveolar	36
Edema, subpleural	21
Congestion, capillary beds	14
Edema, perivascular	13
Hyperinsuflation, alveolar	11
Hemorrhage, subpleural	9

Cont.

Histiocytosis, alveolar	7
Rupture, alveolar	7
Hemorrhage, perivascular	6
Bullae, alveolar	5
Bullae, subpleural	5
Edema, interlobar	5
Pneumonia, interstitial, mononuclear (lymphocytic, histiocytic)	4
Atelectasis	2
Hemorrhage, bronchus	2
Leukocyte infiltration, subpleural, mononuclear (lymphocytic)	2
Agonic aspiration, alveoli	1
Edema, submucosa	1
Hemorrhage, interlobar	1
Hemorrhage, periserosal	1
Hemosiderosis	1
Pneumonia, granulocytic (neutrophilic)	1
Pneumonia, interstitial, granulocytic (neutrophilic), acute	1
Autolysis	2
Hemorrhage, alveolar	49
Lymph node	**21**
Paracortical lymphoid reactive hyperplasia	18
Hemosiderosis	13
Erythrophagocytosis	2
Hemorrhage, sinusal	2
Histiocytosis, sinusal	2
Inclusion, intracytoplasmic, Histiocytic	1
Leukocyte infiltration, medullar, granulocytic (eosinophilic)	1
Lymphadenitis, fibrin hemorrhagic, granulocytic (eosinophilic, neutrophilic)	1
Lymphadenitis, granulocytic (eosinophilic, neutrophilic)	1
Lymphadenitis, granulocytic (eosinophylic)	1
Lymphadenitis, granulocytic (neutrophilic)	1
No significant findings	1
Lymph node, mesenteric	**5**
Lymphadenitis, granulocytic (eosinophilic)	3
Paracortical lymphoid reactive hyperplasia	2
Erythrophagocytosis	1
Histiocytosis, sinusal	1
Inclusion, intracytoplasmic, Histiocytic	1
Leukocytic infiltrate, perivascular, mononuclear (plasmacytic)	1
No significant findings	1
Pancreas	**38**
Hemorrhage, parenchyma	9
Pancreatitis, mononuclear (lymphocytic)	1
No significant findings	25
Autolysis	6
Penis	**1**
No significant findings	1
Peripheral nerve	**1**
No significant findings	1
Salivary gland	**34**
No significant findings	33
Autolysis, advanced	1
Skeletal muscle	**29**
Degeneration, myofiber	8
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	6
Rupture, myofiber	4
Necrosis, myofiber	2

Cont.

	Degeneration/necrosis, myofiber	1
	No significant findings	14
	Autolysis	2
Skin		**18**
	Orthokeratotic hyperkeratosis	3
	Acanthosis	1
	Dermatitis, granulocytic (neutrophilic)	1
	Fasciitis, suppurative, (neutrophilic)	1
	Fibroplasia	1
	Folliculitis, hemorrhagic, mixed (neutrophilic, histiocytic)	1
	Leukocyte infiltration, perivascular, dermis, granulocytic (eosinophilic)	1
	Leukocyte infiltration, perivascular, granulocytic (eosinophylic)	1
	No significant findings	13
Small intestine		**53**
	Enteritis, mixed (lymphocytic, eosinophilic) by nematode larvae	3
	Enteritis, submucosa, granulocytic (eosinophilic)	3
	Metazoan, acanthocephala, adult/eggs, lumen	3
	Enteritis, granulocytic (eosinophylic)	2
	MALT hyperplasia	2
	Edema, submucosa	1
	Enteritis, granulocytic (eosinophylic) by acanthocephala	1
	Enteritis, granulocytic (eosinophylic) by nematodes	1
	Enteritis, mixed (lymphocytic, neutrophilic) with trematode eggs	1
	Enteritis, mononuclear (lymphocytic, histiocytic)	1
	Hemosiderosis	1
	Metazoan, nematode, larvae, adult, lumen	1
	Metazoan, Trematode, larvae, adult, lumen	1
	Metazoan, undetermined	1
	No significant findings	25
	Autolysis	21
Spine		**2**
	No significant findings	2
Spleen		**48**
	Hemorrhage, red pulp	14
	Histiocytosis, red pulp	8
	Hemosiderosis	7
	White pulp depletion	7
	Extramedular hematopoiesis	5
	Reactivity, red pulp	4
	Leukocytic infiltrate, red pulp, granulocytic (eosinophilic)	2
	Splenitis, granulocytic (eosinophilic)	2
	White pulp hyperplasia	2
	Anthracosis	1
	Congestion	1
	Splenitis, granulocytic (eosinophilic, neutrophilic)	1
	No significant findings	13
	Autolysis	4
Stomach		**39**
	Gastritis, granulocytic (eosinophylic)	3
	Gastritis, granulomatous	3
	Gastritis, mononuclear (lymphocytic, plasmacytic, histiocytic)	2
	Metazoan, nematode, larvae, adult, lumen, no inflammation	2
	Arthropod, ant (possible), lumen	1
	Edema, submucosa	1
	Gastritis, submucosal, granulocytic (eosinophilic)	1
	Leiomyositis, granulocytic (eosinophilic)	1
	Leukocyte infiltrate, mucosa/submucosa, granulocytic (eosinophilic)	1

	Metazoan, nematode, larvae, adult, mucosa, larva migrans	1
	No significant findings	21
	Autolysis	8
Testicle		**9**
	Sexual maturity	5
	No significant findings	3
	Autolysis	1
Thymus		**3**
	No significant findings	3
Thyroid		**2**
	No significant findings	2
Tongue		**39**
	Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	11
	Glossitis, internal, mononuclear (histiocytic)	4
	Hemorrhage, muscular layer	2
	Degeneration, myofiber	1
	Glossitis, internal, mixed (neutrophilic, histiocytic, lymphocytic)	1
	Hemorrhage, mucosa	1
	No significant findings	24
Tonsil		**1**
	No significant findings	1
Trachea		**45**
	Hemorrhage, serosa	6
	Hemorrhage, submucosa	6
	Hemorrhage, mucosa	1
	Mineralization, cartilage	1
	Tracheitis, granulocytic (neutrophilic)	1
	No significant findings	31
	Autolysis	1
Urinary bladder		**24**
	Hemorrhage, serosa	2
	Hemorrhage, submucosa	1
	No significant findings	20
	Autolysis	1

Tamandua tetradactyla (n=41)		
Adrenal glands		**36**
	Hemorrhage, pericapsular	4
	Loss/fibrosis, cortex, fascicular/reticular	4
	Vacuolar degeneration, cortex, fascicular	3
	Vacuolar degeneration, cortex, fascicular/reticular	2
	Adrenalitis, cortical, mononuclear (lymphocytic)	1
	Adrenalitis, corticomedular interface, mixed (lymphocytic, neutrophilic)	1
	Congestion, cortical, fascicular	1
	Fibrosis, cortex	1
	Hemorrhage, cortical	1
	Hemorrhage, cortical, fascicular/reticular	1
	Laceration, parenchyma	1
	No significant findings	11
	Autolysis	5
Bone Marrow		**1**
	No significant findings	1
Brain		**10**
	Congestion, leptomeningeal	1
	Edema, perivascular	1
	Hemorrhage	1
	Hemorrhage, leptomeningeal	1
	Vacuolization	1

		Cont.
	No significant findings	5
Carotid		**9**
	No significant findings	9
Cerebellum		**8**
	Hemorrhage, leptomeningeal	1
	No significant findings	7
Diaphragm		**9**
	Protozoa, sarcocystid, Intrasarcolemal, no inflammation	2
	Rupture, myofiber	2
	No significant findings	6
Duodenum		**1**
	Enteritis, submucosa, granulocytic (eosinophilic)	1
	Autolysis	1
Esophagus		**22**
	Hemorrhage, serosa	4
	Degeneration, myofiber	1
	Edema, submucosa	1
	Esophagitis, granulocytic (eosinophilic)	1
	Hemorrhage, periserosal	1
	Metazoan, nematode, larvae, lumen	1
	Necrosis, myofiber	1
	No significant findings	13
	Autolysis	2
Gland		**2**
	No significant findings	2
Heart		**30**
	Degeneration, cardiomyocyte	2
	Leukocytic infiltrate, perivascular, mononuclear (lymphocytic)	2
	Hemorrhage, epicardial	2
	Hemorrhage, myocardial	1
	Myocarditis, mononuclear (lymphocytic)	1
	Pericarditis, granulocytic (eosinophilic)	1
	No significant findings	20
	Autolysis	4
Jugular		**5**
	No significant findings	5
Kidney		**33**
	Proteinosis, tubular	7
	Hemorrhage, cortex	3
	Nephritis, interstitial, mononuclear (lymphocytic)	3
	Mineralization, tubular, medullae	2
	Atrophy, glomeruli	1
	Emboli, hepatic tissue, hepatocytes	1
	Hemorrhage	1
	Proteinosis, glomerular	1
	Proteinosis, tubular/glomerular	1
	No significant findings	13
	Autolysis	9
Large intestine		**14**
	Colitis, mixed (eosinophilic, lymphocytic)	1
	MALT hyperplasia	1
	Protozoa, cyst (Trypanosoma)	1
	Thrombi, metazoan structure	1
	No significant findings	7
	Autolysis	5
Liver		**36**
	Congestion, sinusoidal	5

Cont.

Hepatitis, lobular, mononuclear (lymphocytic, histiocytic)	5
Laceration, parenchyma	3
Extramedular hematopoiesis	2
Hemorrhage, lobular	2
Cholangitis, mononuclear (lymphocytic)	1
Congestion, portal	1
Emboli, hepatic tissue, hepatocytes	1
Granuloma, parasitic	1
Hemorrhage, portal	1
Hepatitis, lobular, granulocytic (neutrophilic)	1
Hepatitis, lobular, mixed (neutrophilic, lymphocytic)	1
Hepatitis, portal, mononuclear (lymphocytic, histiocytic)	1
Leukocyte infiltration, periductular, mononuclear (lymphocytic)	1
Leukocyte infiltration, sinusoids, mixed (neutrophilic, lymphocytic)	1
Metazoan, nematode, microfilaries, intravascular, no inflammation	1
Pericholangitis, mononuclear (lymphocytic)	1
No significant findings	15
Lungs	**39**
Edema, alveolar	34
Edema, perivascular	25
Hemorrhage, alveolar	24
Hemorrhage, perivascular	15
Bullae, subpleural	14
Hyperinsuflation, alveolar	11
Bullae, alveolar	9
Congestion, capillary beds	8
Edema, subpleural	7
Hemorrhage, subpleural	6
Rupture, alveolar	6
Emboli, bone narrow	4
Hemorrhage, interlobar	4
Hemosiderosis	2
Histiocytosis, alveolar	2
Pneumonia, granulomatous with MNGC	2
Pneumonia, interstitial, mononuclear (histiocytic)	2
Congestion, vascular	1
Edema, interlobar	1
Edema, submucosa	1
Emboli, adipose tissue	1
Emboli, hepatic tissue, hepatocytes	1
Granuloma, parasitic	1
Hemorrhage, bronchus	1
Leukocyte infiltration, perivascular, mononuclear (lymphocytic, histiocytic)	1
Metazoan, nematode, microfilaries, intravascular, no inflammation	1
Metazoan, undetermined, subpleural interstitium,	1
Parasitic granuloma by cestode	1
Autolysis	4
Lymph node	**19**
Paracortical lymphoid reactive hyperplasia	8
Hemorrhage, sinusal	3
Lymphadenitis, granulocytic (eosinophilic)	2
Hemorrhage, pericapsular	1
Hemosiderosis	1
Histiocytosis, sinusal	1
No significant findings	7
Autolysis	3

Cont.

Lymph node, mesenteric **7**

Paracortical lymphoid reactive hyperplasia	5
Lymphadenitis, granulocytic (eosinophilic)	2
Hemorrhage, sinusal	1
No significant findings	1
Autolysis, moderate	1

Nerve **1**

No significant findings	1

Ovary **2**

No significant findings	2

Pancreas **22**

Hemorrhage, parenchyma	2
Laceration, parenchyma	1
Metazoan, nematode, larvae, adult, duct	1
No significant findings	13
Autolysis	6

Salivary gland **13**

No significant findings	11
Autolysis	2

Skeletal muscle **16**

Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	6
Rupture, myofiber	4
Degeneration, myofiber	2
Degeneration/necrosis, myofiber	1
Hemorrhage, interstitial	1
No significant findings	7

Skin **14**

Arthropod, tick, Sagittal cut	1
Dermatitis, granulocytic (neutrophilic) by tick	1
No significant findings	12
Autolysis	1

Small intestine **30**

Enteritis, mixed (lymphocytic, histiocytic, eosinophylic) with intraluminal nematode larvae	6
Enteritis, mononuclear (lymphocytic, histiocytic)	5
Protozoa, Eimeria, crypta	5
Enteritis, granulocytic (eosinophylic)	3
Metazoan, acanthocephala, larvae, adult, lumen	3
Metazoan, nematode, larvae, adult, lumen	3
Hemorrhage, submucosa	1
MALT hyperplasia	1
No significant findings	6
Autolysis	16

Spleen **30**

Hemorrhage, red pulp	7
White pulp hyperplasia	5
Extramedular hematopoiesis	3
Hemosiderosis	3
Histiocytosis, red pulp	3
White pulp depletion	2
Congestion	1
Hemorrhage, serosa	1
Leukocytic infiltrate, red pulp, granulocytic (eosinophilic)	1
Splenitis, granulocytic (neutrophilic)	1
No significant findings	9
Autolysis	9

	Cont.
Stomach	**21**
Gastritis, granulocytic (eosinophylic)	5
Gastritis, mixed (eosinophylic, lymphocytic)	3
Gastritis, granulomatous	1
Gastritis, submucosal, granulocytic (eosinophilic)	1
Hemorrhage, serosa	1
Leiomyositis, mixed (histiocytic, eosinophilic) by metazoan larvae	1
Metazoan, nematode, ascarid, larvae, adult, lumen	1
Metazoan, nematode, larvae, tunica muscularis, intravascular	1
No significant findings	10
Autolysis	2
Testicle	**11**
Sexual maturity	7
Orquitis, mononuclear (lymphocytic)	1
No significant findings	1
Autolysis	3
Thymus	**1**
No significant findings	1
Thyroid	**1**
Autolysis	1
Tongue	**21**
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	7
Glossitis, mononuclear (lymphocytic)	1
Glossitis, superficial, mixed (eosinophilic, lymphocytic)	1
No significant findings	12
Trachea	**25**
Hemorrhage, serosa	5
Hemorrhage, submucosa	2
Hemorrhage, mucosa	1
Leukocytic infiltrate, submucosal, mononuclear (lymphocytic)	1
No significant findings	19
Urinary bladder	**15**
No significant findings	13
Autolysis	2
Uterus	**5**
No significant findings	5

APPENDIX VIII

General information, gross and histopathological findings in non-human primates.

Table 1. General information of non-human primates.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-004	*Alouatta caraya*	Female	Juvenile	4,1	Regular	2016	November	Rain	SP	Itapecerica da Serra	Atlantic forest
RK-038	*Alouatta caraya*	Male	Juvenile		Regular	2017	July	Dry	SP	Caraguatatuba	Atlantic forest
RK-051	*Alouatta caraya*	Female	Juvenile	4	Good	2017	October	Rain	SP	Caraguatatuba	Atlantic forest
RK-054	*Alouatta caraya*	Male	Juvenile	2,3	Regular	2018	January	Rain	SP	Itapecerica da Serra	Atlantic forest
RK-055	*Alouatta guariba clamitans*	Male	Adult	6,7	Good	2015	August	Dry	SP	Jacupiranga	Atlantic forest
RK-056	*Alouatta guariba clamitans*	Male	Adult	6,6	Good	2016	June	Dry	SP	Itapecerica da Serra	Atlantic forest
RK-058	*Alouatta caraya*	Female	Juvenile	4,1	Good	2017	November	Rain	SP	Caraguatatuba	Atlantic forest
RK-091	*Alouatta guariba clamitans*	Male	Adult	6,6	Regular	2017	March	Rain	SP	Jacupiranga	Atlantic forest
RK-313	*Alouatta guariba clamitans*	Male	Adult	6,2	Good	2018	September	Dry	SP	Embu	Atlantic forest
RK-327	*Alouatta guariba clamitans*	Male	Adult	6,6	Good	2019	April	Dry	SC	Itaiópolis	Atlantic forest
RK-331	*Alouatta guariba clamitans*	Male	Juvenile	2,3	Good	2018	October	Rain	SC	Itaiópolis	Atlantic forest
RK-358	*Alouatta guariba clamitans*	Male	Adult	7,48	Good	2016	February	Rain	PR	Piraquara	Atlantic forest
RK-041	*Callithrix aurita*	Male	Juvenile	0,31	Regular	2017	June	Dry	SP	Jambeiro	Atlantic forest
RK-064	*Callithrix aurita*	Female	Adult	0,41	Good	2017	November	Rain	SP	São José dos Campos	Atlantic forest
RK-307	*Callithrix aurita*	Female	Adult	0,48	Good	2018	November	Rain	SP	Jambeiro	Atlantic forest
RK-424	*Callithrix aurita*	Male	Adult	0,42	Good	2019	May	Dry	SP	São José dos Campos	Atlantic forest
RK-245	*Sapajus libidinosus*	Female	Juvenile	1	Good	2018	July	Dry	MS	Aquidauana	Pantanal
RK-246	*Sapajus libidinosus*	Female	Infant	0,7	Good	2018	July	Dry	MS	Miranda	Pantanal
RK-337	*Sapajus nigritus*	Male	Adult	3,77	Good	2011	August	Dry	SC	São Francisco do Sul	Atlantic forest
RK-346	*Sapajus nigritus*	Male	Adult	3,59	Good	2014	August	Dry	SC	Garuva	Atlantic forest

Figure 2. Spatial distribution, and main characteristics of non-human primates.
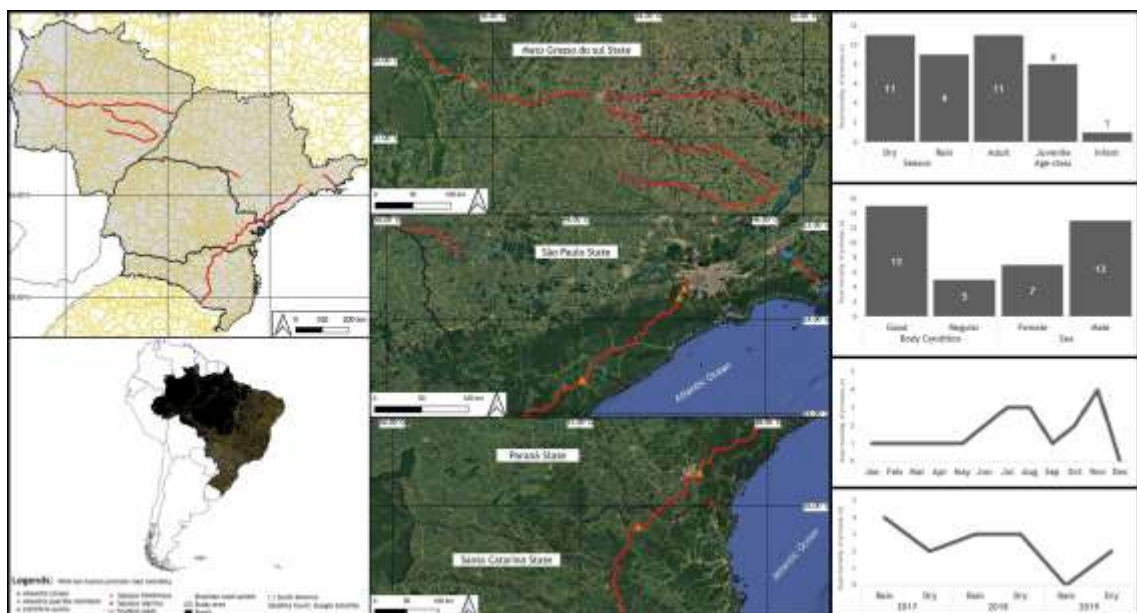
Table 2. Gross findings non associated with trauma in non-human primates.

Species, gross finding	Cases
Alouatta caraya (n=5)	
Splenomegaly	40% (2/5)
Small intestine, endoparasitic infestation	40% (2/5)
Epicardial parasitic migration	20% (1/5)
Splenic white pulp hyperplasia	20% (1/5)
Large intestine, endoparasitic infestation	20% (1/5)
Lice infestation	20% (1/5)
Lymph node megaly	20% (1/5)
Regular body condition	20% (1/5)
Stomach, endoparasitic infestation	20% (1/5)
Alouatta guariba clamitans (n=7)	
Lice infestation	29% (2/7)
Large intestine, endoparasitic infestation	14% (1/7)
Oral cavity, papilloma	14% (1/7)
Callithrix aurita (n=4)	
Adrenomegaly	25% (1/4)
Mesenteric lymph node megaly	25% (1/4)
Small intestine, endoparasitic infestation	25% (1/4)
Sapajus libidinosus (n=2)	
Splenic white pulp hyperplasia	100% (2/2)
Mesenteric lymph node megaly	50% (1/2)
Sapajus nigritus (n=2)	
Small intestine, endoparasitic infestation	100% (1/1)

Figure 2. Main gross findings in non-human primates.
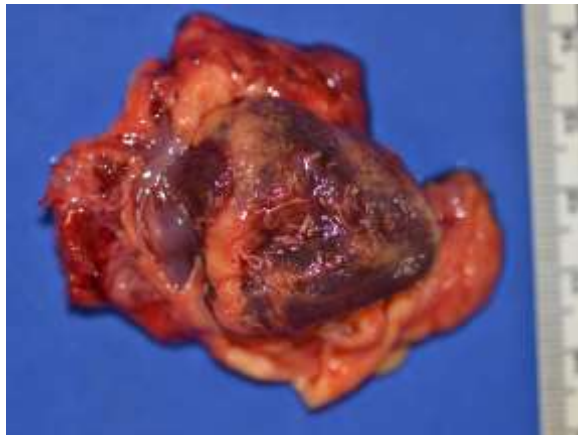
RK-054. *Alouatta caraya*. Male, juvenile. Epicardial nematode.

RK-313. *Alouatta guariba clamitans*. Male, adult. Genvival papilloma.

RK-091. *Alouatta guariba clamitans*. Male, adult. Lice, genus *Cebidicola* spp.

RK-065. *Aouatta guariba clamitans*. Male, adult. Lice, genus *Cebidicola* spp.

Table 3. Microscopic findings of non-human primates. Black howler monkey (*Alouatta caraya*, n=5), brown howler monkey (*Alouatta guariba clamitans*, n=7), white-eared marmoset (*Callithrix aurita*, n=4), bearded capuchin (*Sapajus libidinosus*, n=2), black bearded capuchin (*Sapajus nigritus*, n=2).

Species, microscopic findings	N° cases
***Alouatta caraya* (n=5)**	
Adrenal glands	**2**
Adrenalitis, mixed (lymphocytic, neutrophilic)	1
Vacuolar degeneration, cortex	1
Bone Marrow	**1**
No significant findings	1
Brain	**3**
No significant findings	2
Autolysis	1
Carotid	**1**
No significant findings	1
Cerebellum	**2**
No significant findings	1
Autolysis	1
Diaphragm	**1**
No significant findings	1
Esophagus	**1**
No significant findings	1
Heart	**5**
Parasitic granuloma, epicardium	1
No significant findings	4
Kidney	**5**
Glomerulosclerosis	2
Nephritis, interstitial, mononuclear (lymphocytic)	1
Nephritis, interstitial, mononuclear (lymphocytic, histiocytic)	1
No significant findings	1
Autolysis	2
Large intestine	**1**
No significant findings	1

Liver	**5**
Hepatitis, lobular, mononuclear (lymphocytic, histiocytic)	4
Degeneration, hydropic, hepatocyte	1
Degeneration, steatosis, macrogoticular, hepatocyte	1
Pericholangitis, mononuclear (lymphocytic, histiocytic)	1
No significant findings	1
Autolysis	1
Lungs	**4**
Edema, alveolar	4
Hemorrhage, alveolar	4
Edema, perivascular	3
Hemosiderosis	3
Agonic aspiration, alveoli, foreign material, vegetal fiber	1
Edema, subpleural	1
Hemorrhage, perivascular	1
Hyperinsuflation, alveolar	1
Leukocyte infiltration, perivascular, mononuclear (lymphocytic, histiocytic)	1
Pneumonia, interstitial, mixed (eosinophylic, lymphocytic)	1
Pneumonia, subpleural, granulocytic (neutrophilic)	1
Pneumonia, subpleural, granulocytic (neutrophilic) with thrombosis	1
Lymph node	**2**
Hemosiderosis	1
No significant findings	1
Lymph node, mesenteric	**1**
No significant findings	1
Ovary	**1**
No significant findings	1
Penis	**1**
No significant findings	1
Salivary gland	**1**
No significant findings	1
Skeletal muscle	**4**
Degeneration, myofiber	2
Rupture, myofiber	2
No significant findings	1
Skin	**2**
Dermatitis, fasciitis, periodicities, mixed (neutrophilic, histiocytic, lymphocytic)	1
No significant findings	1
Small intestine	**4**
Enteritis, mixed (lymphocytic, eosinophilic) by nematode larvae	1
MALT hyperplasia	1
Metazoan, nematode, ascarid, larvae, adult, lumen	1
No significant findings	1
Autolysis	1
Spleen	**4**
Hemosiderosis	2
Anthracosis	1
Extramedular hematopoiesis	1
Histiocytosis, red pulp	1
White pulp hyperplasia	1
No significant findings	1

Cont.

Stomach	**2**
No significant findings	2
Testicle	**1**
No significant findings	1
Tongue	**3**
Glossitis, internal, mixed (neutrophilic, histiocytic, lymphocytic)	1
Metazoan, nematode, Capillaria, larvae, adult and eggs, epithelium	1
No significant findings	1
Trachea	**3**
No significant findings	3
Urinary bladder	**3**
No significant findings	3
Uterus	**1**
No significant findings	1

***Alouatta guariba clamitans* (n=7)**	
Adrenal glands	**5**
Hemorrhage, pericapsular	1
No significant findings	2
Autolysis	2
Brain	**2**
Autolysis	2
Carotid	**2**
No significant findings	2
Cerebellum	**1**
Autolysis	1
Diaphragm	**2**
No significant findings	2
Epiglottis	**2**
No significant findings	2
Esophagus	**5**
Leiomyositis, mixed (histiocytic, neutrophilic)	1
No significant findings	4
Heart	**6**
No significant findings	4
Autolysis	2
Jugular	**2**
No significant findings	2
Kidney	**7**
Nephritis, interstitial, mononuclear (lymphocytic)	2
Nephritis, interstitial, mixed (neutrophilic, histiocytic)	1
Autolysis	5
Large intestine	**2**
No significant findings	1
Autolysis	1
Liver	**7**
Hemosiderosis	2
Hepatitis, lobular, granulocytic (neutrophilic)	2
Hepatitis, lobular, mixed (neutrophilic, histiocytic)	1
Hepatitis, lobular, mononuclear (lymphocytic)	1
Hepatitis, portal, mixed (lymphocytic, neutrophilic)	1
Leukocyte infiltration, periductular, mononuclear (lymphocytic)	1
Pericholangitis, mononuclear (lymphocytic)	1
Autolysis	2

		Cont.
Lungs		**7**
	Edema, alveolar	6
	Edema, perivascular	6
	Hemorrhage, alveolar	3
	Anthracosis	1
	Bullae, alveolar	1
	Hemosiderosis	1
	Hyperinsuflation, alveolar	1
	Rupture, alveolar	1
	Autolysis	4
Lymph node		**1**
	No significant findings	1
Pancreas		**1**
	Autolysis	1
Penis		**1**
	No significant findings	1
Skeletal muscle		**3**
	Hemorrhage, interstitial	2
	No significant findings	1
Skin		**5**
	No significant findings	5
Small intestine		**3**
	Metazoan, nematode, larvae, adult, lumen	1
	No significant findings	2
	Autolysis	1
Spleen		**5**
	No significant findings	2
	Autolysis	3
Stomach		**1**
	No significant findings	1
Testicle		**5**
	Sexual maturity	5
Tongue		**3**
	Glossitis, granulocytic (neutrophilic)	1
	No significant findings	2
Trachea		**4**
	No significant findings	4
Urinary bladder		**6**
	No significant findings	4
	Autolysis	4

Callithrix aurita (n=4)

Adrenal glands		**1**
	No significant findings	1
Brain		**1**
	No significant findings	1
Carotid		**1**
	No significant findings	1
Cerebellum		**1**
	No significant findings	1
Esophagus		**2**
	No significant findings	2
Heart		**4**
	No significant findings	4

	Cont.
Jugular	**1**
No significant findings	1
Kidney	**1**
No significant findings	1
Liver	**3**
Hepatitis, lobular, mononuclear (lymphocytic)	1
Hepatitis, portal, mononuclear (lymphocytic, histiocytic)	1
No significant findings	1
Lungs	**4**
Edema, alveolar	4
Edema, perivascular	3
Hemorrhage, alveolar	3
Leukocyte infiltration, perivascular, mononuclear (lymphocytic, histiocytic)	2
Artery, tunica media, hypertrophy	1
Emboli, bone narrow	1
Hemorrhage, bronchus	1
Hemorrhage, peribronchial	1
Hemorrhage, perivascular	1
Metazoan, nematode, microfilaries, intravascular, no inflammation	1
Pneumonia, granulocytic (neutrophilic)	1
Lymph node	**1**
No significant findings	1
Autolysis	1
Salivary gland	**1**
No significant findings	1
Skeletal muscle	**2**
No significant findings	2
Small intestine	**2**
Metazoan, undetermined	1
No significant findings	2
Stomach	**2**
No significant findings	2
Testicle	**1**
Sexual maturity	1
Thyroid	**3**
No significant findings	3
Tongue	**3**
No significant findings	3
Trachea	**3**
No significant findings	3
Uterus	**1**
No significant findings	1
***Sapajus libidinosus* (n=2)**	
Adrenal glands	**2**
Hemorrhage, cortical	1
Hemorrhage, pericapsular	1
No significant findings	1
Brain	**2**
Gliosis	1
Hemorrhage	1
Hemorrhage, leptomeningeal	1
Neuron, hipereosinofilia	1

Cont.

Cerebellum	**2**
No significant findings	2
Esophagus	**2**
No significant findings	2
Heart	**2**
Hemorrhage, perivascular	2
Hemorrhage, myocardial	1
Metazoan, nematode, microfilaries, intravascular, no inflammation	1
Myocarditis, mixed (lymphocytic, eosinophilic)	1
Myocarditis, mononuclear (lymphocytic, histiocytic)	1
Kidney	**2**
Congestion, cortex	1
Nephritis, interstitial, mononuclear (lymphocytic)	1
No significant findings	1
Liver	**2**
Cholangitis, mononuclear (lymphocytic)	1
Degeneration, hydropic, hepatocyte	1
Hemorrhage, lobular	1
Hepatitis, lobular, mononuclear (lymphocytic)	1
Hepatitis, lobular, mononuclear (lymphocytic, histiocytic) by intralesional microfilaria	1
Pericholangitis, mononuclear (lymphocytic, histiocytic)	1
Lungs	**2**
BALT hyperplasia	2
Edema, perivascular	2
Hemorrhage, alveolar	2
Hemorrhage, perivascular	2
Bronchitis, mixed (eosinophilic, histiocytic, lymphocytic) by nematodes	1
Edema, alveolar	1
Pneumonia, granulomatous by lungworm	1
Pneumonia, interstitial, mixed (eosinophylic, lymphocytic)	1
Pneumonia, interstitial, mixed (lymphocytic, histiocytic, neutrophilic) by microfilaries larvae	1
Lymph node	**2**
Metazoan, nematode, microfilaries, intravascular, no inflammation	1
Paracortical lymphoid reactive hyperplasia	2
Pancreas	**1**
Metazoan, nematode, larvae, adult, duct	1
Pituitary	**1**
No significant findings	1
Skin	**1**
No significant findings	1
Small intestine	**2**
Enteritis, mononuclear (Lymphocytic)	1
Protozoa, Eimeria, crypta	1
Spleen	**2**
White pulp hyperplasia	2
Stomach	**2**
Gastritis, granulomatous	1
Gastritis, mononuclear (lymphocytic, histiocytic)	1
No significant findings	1
Thymus	**1**
No significant findings	1

Cont.

Tongue	**1**
Glossitis, internal, mononuclear (histiocytic, lymphocytic)	1
Trachea	**2**
No significant findings	2

***Sapajus nigritus* (n=2)**

Carotid	**2**
No significant findings	2
Esophagus	**2**
No significant findings	2
Heart	**2**
No significant findings	2
Jugular	**2**
No significant findings	2
Lungs	**2**
Edema, perivascular	1
Autolysis	2
Testicle	**1**
Sexual maturity	1
Trachea	**2**
No significant findings	2

APPENDIX IX

General information, gross and histopathological findings in rodents.

Table 1. General information of rodents.

Case	Specie	Sex	Age	Weight (kg)	Body Condition	Year	Month	Season	State	Municipality	Biome
RK-020	*Cavia aperea*	Female	Adult	0,9	Good	2017	June	Dry	SP	Paraibuna	Atlantic forest
RK-045	*Cavia aperea*	Male	Adult	0,6	Good	2017	October	Rain	MS	Rio Brilhante	Cerrado
RK-047	*Cavia aperea*	Female	Adult	0,48	Good	2017	October	Rain	MS	Terenos	Cerrado
RK-075	*Cavia aperea*	Female	Adult	0,64	Good	2017	December	Rain	MS	Campo Grande	Cerrado
RK-200	*Cavia aperea*	Male	Adult	-	Good	2018	March	Rain	MS	Anastácio	Cerrado
RK-363	*Cavia aperea*	Male	Juvenile	0,26	Good	2012	October	Rain	SC	Jaraguá do Sul	Atlantic forest
RK-007	*Coendou spinosus*	Female	Juvenile	1,3	Regular	2016	January	Rain	SP	Registro	Atlantic forest
RK-009	*Coendou spinosus*	Male	Juvenile	1,5	Regular	2015	February	Rain	SP	Miracatu	Atlantic forest
RK-015	*Coendou spinosus*	Male	Adult	1,6	Good	2016	March	Rain	SP	Juquitiba	Atlantic forest
RK-049	*Coendou spinosus*	Female	Juvenile	2,05	Good	2017	September	Dry	SP	Paraibuna	Atlantic forest
RK-262	*Coendou spinosus*	Male	Juvenile	1,4	Good	2018	September	Dry	SP	Paraibuna	Atlantic forest
RK-275	*Coendou spinosus*	Unknown	Juvenile	1	Good	2018	September	Dry	SP	Embu	Atlantic forest
RK-285	*Coendou spinosus*	Female	Juvenile	1,2	Good	2018	December	Rain	SP	Piraju	Cerrado
RK-370	*Coendou spinosus*	Male	Adult	1,5	Good	2016	November	Rain	SC	Itaiópolis	Atlantic forest
RK-176	*Cuniculus paca*	Male	Adult	5,8	Good	2018	March	Rain	SP	Jambeiro	Atlantic forest
RK-076	*Dasyprocta azarae*	Male	Adult	3,14	Good	2017	December	Rain	MS	Miranda	Pantanal
RK-377	*Dasyprocta azarae*	Male	adult	3,19	Good	2017	November	Rain	PR	Mandirituba	Atlantic forest
RK-419	*Guerlinguetus brasiliensis*	Male	Adult	0,17	Good	2019	June	Dry	SP	Paraibuna	Atlantic forest
RK-021	*Hydrochoerus hydrochaeris*	Male	Adult	30,2	Good	2017	January	Rain	SP	Paraibuna	Atlantic forest
RK-022	*Hydrochoerus hydrochaeris*	Female	Adult	32,3	Good	2017	January	Rain	SP	Paraibuna	Atlantic forest
RK-033	*Hydrochoerus hydrochaeris*	Male	Juvenile	22,5	Good	2017	July	Dry	SP	Jambeiro	Atlantic forest
RK-060	*Hydrochoerus hydrochaeris*	Male	Adult	35	Good	2017	November	Rain	MS	Aquidauana	Pantanal
RK-065	*Hydrochoerus hydrochaeris*	Male	Adult	32,3	Good	2017	November	Rain	SP	Paraibuna	Atlantic forest
RK-069	*Hydrochoerus hydrochaeris*	Female	Juvenile	18	Good	2017	December	Rain	MS	Anastácio	Cerrado
RK-174	*Hydrochoerus hydrochaeris*	Female	Adult	65,0	Good	2017	December	Rain	SP	Paraibuna	Atlantic forest
RK-235	*Hydrochoerus hydrochaeris*	Male	Adult	64	Good	2017	December	Rain	SP	Paraibuna	Atlantic forest
RK-257	*Hydrochoerus hydrochaeris*	Male	Adult	61	Good	2018	August	Dry	SP	São José dos Campos	Atlantic forest
RK-308	*Hydrochoerus hydrochaeris*	Female	Adult	49	Good	2019	February	Rain	SP	Paraibuna	Atlantic forest
RK-409	*Hydrochoerus hydrochaeris*	Male	Adult	45,2	Good	2019	June	Dry	SP	Paraibuna	Atlantic forest
RK-441	*Hydrochoerus hydrochaeris*	Male	Adult	25	Good	2019	December	Rain	MS	Anastácio	Cerrado
RK-353	*Kannabateomys amblyonyx*	Male	Adult	0,32	Good	2014	April	Dry	PR	Tijucas do Sul	Atlantic forest
RK-368	*Myocastor coypus*	Male	Adult	3,58	Good	2016	November	Rain	SC	Papanduva	Atlantic forest
RK-411	*Myocastor coypus*	Male	Adult	3,4	Good	2019	June	Dry	SP	Itapecerica da Serra	Atlantic forest
RK-364	*Rattus norvegicus*	Male	Adult	0,19	Good	2014	July	Dry	SC	Governador Celso Ramos	Atlantic forest
RK-365	*Rattus norvegicus*	Female	Adult	0,25	Good	2018	June	Dry	SC	Santa Cecília	Atlantic forest

Figure 1. Spatial distribution, and main characteristics of rodents.
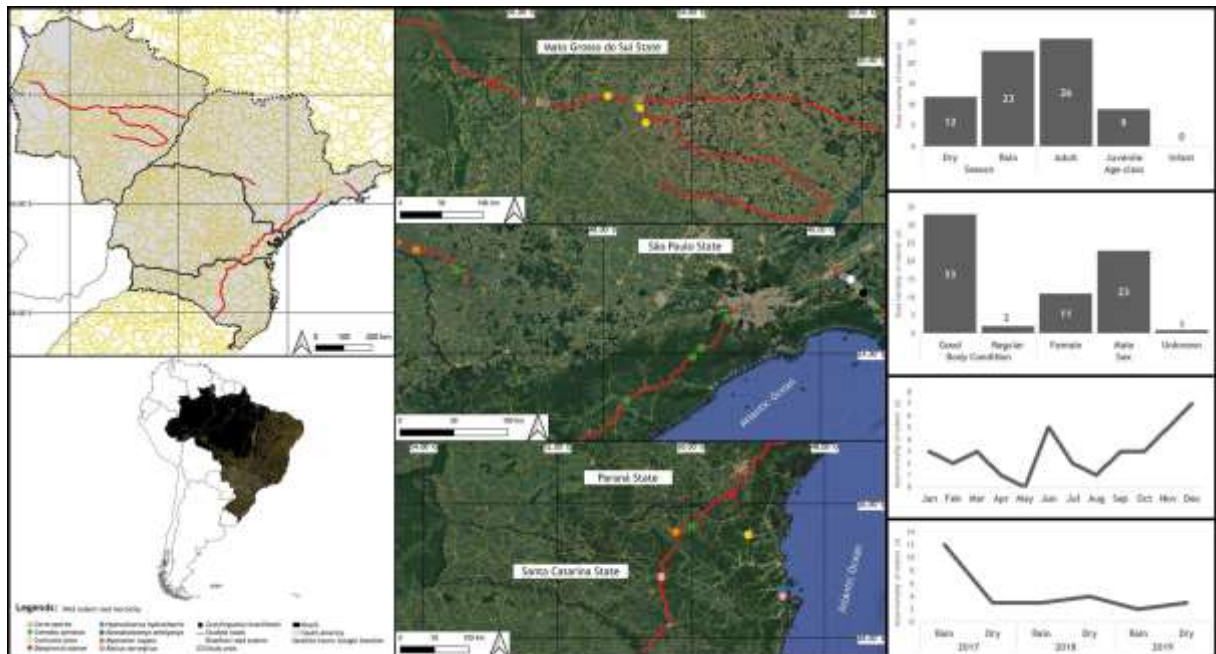
Table 2. Gross findings non associated with trauma in the rodents.

Species, gross finding	Cases
***Cavia aperea* (n=6)**	
Lymph node megaly	17% (1/6)
Pregnancy	17% (1/6)
***Coendou spinosus* (n=8)**	
Lice infestation	75% (6/8)
Tick infestation	50% (4/8)
Splenomegaly	13% (1/8)
Dasyprocta azarae (n=2)	
Splenic white pulp hyperplasia	50% (1/2)
Predation	50% (1/2)
Hydrochoerus hydrochaeris (n=12)	
Tick infestation	58% (7/12)
Esophageal ulcer	8% (1/12)
Splenic white pulp hyperplasia	8% (1/12)
Large intestine, endoparasitic infestation	8% (1/12)
Passive hepatic congestion	8% (1/12)
Myocastor coypus (n=2)	
Large intestine, endoparasitic infestation	50% (1/2)
Megaly of tonsil	50% (1/2)
Tick infestation	50% (1/2)

Figure 2. Main gross findings.

RK-257. *Hydrochoerus hydrochaeris*. Male, adult. Tick, Family: *Ixodidae*, genus: *Amblyomma*.

RK-262. *Coendous spinosus*. Male, juvenile. Tick, Family: *Ixodidae*, genus: *Amblyomma*.

RK-262. *Coendou spinosus*. Male, juvenile. Lice. Genus: *Eutrichophilus* spp.

Table 3. Microscopic findings of rodents. Brazilian Guinea Pig (*Cavia aperea*, n=6), hairy dwarf porcupine (*Coendou spinosus*, n=8), paca (*Cuniculus paca*, n=1), Azara's agouti (*Dasyprocta azarae*, n=2), capybara (*Hydrochoerus hydrochaeris*, n=12), coypu (*Myocastor coypus*, n=2), brown rat (*Rattus norvegicus*, n=1), Brazilian Squirrel (*Guerlinguetus brasiliensis*, n=1).

Species, microscopic findings	N° cases
***Cavia aperea* (n=6)**	
Adrenal glands	**3**
Adrenalitis, cortical, mononuclear (lymphocytic)	1
Vacuolar degeneration, cortex	1
No significant findings	2
Brain	**1**
No significant findings	1
Carotid	**3**
No significant findings	3

Cont.

Diaphragm	**2**
No significant findings	2
Epiglottis	**1**
Protozoa, sarcocystid, Intrasarcolemal, no inflammation	1
Esophagus	**3**
No significant findings	3
Eye	**1**
No significant findings	1
Heart	**4**
Leukocytic infiltrate, perivascular, mixed (lymphocytic, neutrophilic)	2
Hemorrhage, pericardial	1
Myocarditis, mononuclear (lymphocytic)	1
No significant findings	1
Jugular	**3**
No significant findings	3
Kidney	**4**
Fibrosis with capsule retraction	1
Glomerulosclerosis	1
Mineralization, tubular, medullae	1
Nephritis, interstitial, mononuclear (lymphocytic)	1
Proteinosis, tubular	1
No significant findings	1
Large intestine	**1**
No significant findings	1
Liver	**4**
Cholangiohepatitis, mononuclear (lymphocytic, histiocytic)	1
Degeneration, hydropic, hepatocyte	1
Degeneration, steatosis, macrogoticular, hepatocyte	1
Hepatitis, lobular/portal, mixed (neutrophilic, histiocytic)	1
Laceration, parenchyma	1
Pericholangitis, mononuclear (lymphocytic, histiocytic)	1
Lungs	**4**
Edema, alveolar	3
Artery, tunica media, hypertrophy	2
Hemorrhage, alveolar	2
BALT hyperplasia	1
Bronchitis, granulomatous	1
Leukocyte infiltration, perivascular, mononuclear (lymphocytic, histiocytic)	1
No significant findings	1
Ovary	**1**
No significant findings	1
Pancreas	**1**
No significant findings	1
Skeletal muscle	**1**
Rupture, myofiber	1
Small intestine	**4**
No significant findings	4
Spleen	**3**
Hemosiderosis	2
No significant findings	1
Stomach	**3**
No significant findings	3
Testicle	**1**
Sexual maturity	1
Tongue	**1**
Protozoa, sarcocystid, intrasarcolemal cyst, no inflammation	1

Cont.

Trachea		**4**
	No significant findings	4
Urinary bladder		**1**
	No significant findings	1

***Coendou spinosus* (n=8)**

Adrenal glands		**1**
	Autolysis	1
Brain		**2**
	No significant findings	1
	Autolysis	1
Carotid		**1**
	No significant findings	1
Diaphragm		**1**
	Autolysis	1
Esophagus		**4**
	No significant findings	3
	Autolysis	1
Heart		**7**
	No significant findings	6
	Autolysis	1
Jugular		**2**
	Hemorrhage, perivascular	1
	No significant findings	1
Kidney		**7**
	No significant findings	2
	Autolysis	5
Large intestine		**1**
	Autolysis	1
Liver		**7**
	Hepatocytes, Intranuclear inclusion	1
	No significant findings	2
	Autolysis	5
Lungs		**7**
	Edema, alveolar	7
	Hemorrhage, alveolar	6
	Edema, perivascular	5
	Congestion, capillary beds	2
	Hemorrhage, perivascular	2
	Hyperinsuflation, alveolar	2
	Metazoan, nematode, microfilaries, intravascular	2
	Hemosiderosis	1
	Autolysis	1
Lymph node		**1**
	No significant findings	1
Pancreas		**1**
	Autolysis	1
Penis		**2**
	No significant findings	2
Salivary gland		**1**
	Autolysis	1
Skeletal muscle		**4**
	Rupture, myofiber	1
	No significant findings	3
Skin		**1**
	No significant findings	1
Small intestine		**5**
	No significant findings	3

Cont.

	Autolysis	4
Spleen		**6**
	Hemorrhage, red pulp	1
	Hemosiderosis	1
	Autolysis	4
Testicle		**3**
	Sexual maturity	2
	Azoospermia	1
Thymus		**2**
	No significant findings	2
Thyroid		**1**
	No significant findings	1
Tongue		**4**
	No significant findings	4
Trachea		**5**
	No significant findings	4
	Autolysis	1
Urinary bladder		**2**
	No significant findings	2

Cuniculus paca (n=1)

Adrenal glands		**1**
	No significant findings	1
Diaphragm		**1**
	No significant findings	1
Esophagus		**1**
	No significant findings	1
Heart		**1**
	Leukocytic infiltrate, perivascular, granulocytic (neutrophilic)	1
Kidney		**1**
	Autolysis	1
Liver		**1**
	Degeneration, hydropic, hepatocyte	1
	Hepatitis, lobular, mixed (neutrophilic, lymphocytic)	1
Lungs		**1**
	Congestion, capillary beds	1
	Edema, alveolar	1
	Edema, perivascular	1
	Hemorrhage, alveolar	1
	Leukocyte infiltration, perivascular, mononuclear (lymphocytic, histiocytic)	1
	Pneumonia, granulocytic (neutrophilic)	1
Spleen		**1**
	Histiocytosis, red pulp	1
Testicle		**1**
	No significant findings	1
Tongue		**1**
	No significant findings	1

Dasyprocta azarae (n=2)

Carotid		**1**
	No significant findings	1
Esophagus		**1**
	No significant findings	1
Heart		**2**
	No significant findings	2
Jugular		**1**
	No significant findings	1

Cont.

Kidney	**2**
Hemorrhage, pericapsular	1
Autolysis	1
Liver	**1**
Hepatitis, lobular, mononuclear (lymphocytic)	1
Lungs	**2**
Edema, alveolar	2
Edema, perivascular	2
Bullae, alveolar	1
Bullae, subpleural	1
Congestion, capillary beds	1
Hemorrhage, alveolar	1
Hemorrhage, perivascular	1
Hemorrhage, subpleural	1
Hemosiderosis, peribronchial	1
Leukocyte infiltration, perivascular, mononuclear (lymphocytic)	1
Pneumonia, pyogranulomatous by adiaspores (Emmonsia)	1
Lymph node	**1**
No significant findings	1
Skeletal muscle	**1**
No significant findings	1
Small intestine	**1**
No significant findings	1
Spleen	**2**
Hemorrhage, red pulp	1
White pulp hyperplasia	1
Autolysis	1
Testicle	**2**
Sexual maturity	2
Trachea	**1**
No significant findings	1

***Hydrochoerus hydrochaeris* (n=12)**

Adrenal glands	**4**
Cortical hemorrhage	1
No significant findings	1
Autolysis	3
Brain	**5**
Protozoa, ciliates, Intrapreneurial	1
No significant findings	2
Autolysis	2
Carotid	**5**
Thrombosis	1
No significant findings	4
Cerebellum	**1**
No significant findings	1
Diaphragm	**5**
No significant findings	5
Esophagus	**7**
Protozoa, ciliates, serosa	1
No significant findings	6
Heart	**10**
Protozoa, ciliated, intraventricular	1
No significant findings	9
Jugular	**2**
No significant findings	2
Kidney	**9**
Nephritis, interstitial, mononuclear (lymphocytic)	3

Atrophy, glomeruli	1	
Hemorrhage, cortex, glomerular	1	
Pyelitis, mixed (neutrophilic, lymphocytic)	1	
No significant findings	1	
Autolysis	5	
Large intestine	**1**	
Metazoan, Trematode, larvae, adult, lumen	1	
Liver	**10**	
Hepatitis, lobular, mixed (neutrophilic, lymphocytic)	2	
Protozoa, ciliates, sinusoids, no inflammation	2	
Hepatitis, lobular, mononuclear (lymphocytic)	1	
Hepatitis, portal, mononuclear (lymphocytic, histiocytic)	1	
Thrombus, fibrin cellular, occluding hepatic arteriole	1	
No significant findings	1	
Autolysis	5	
Lungs	**11**	
Edema, alveolar	7	
Edema, perivascular	5	
Hemorrhage, alveolar	5	
Pneumonia, granulocytic (neutrophilic)	4	
Protozoa, ciliates	3	
Hemosiderosis	2	
Pneumonia, interstitial, granulocytic (neutrophilic), acute	2	
Hemorrhage, bronchus	1	
Histiocytosis, alveolar	1	
Pneumonia, granulomatous with MNGC	1	
Rupture, alveolar	1	
Autolysis	1	
Lymph node	**3**	
No significant findings	2	
Autolysis, advanced	2	
Lymph node, cervical	**1**	
No significant findings	1	
Pancreas	**2**	
No significant findings	1	
Autolysis	1	
Penis	**1**	
No significant findings	1	
Skeletal muscle	**6**	
Degeneration, myofiber	3	
Rupture, myofiber	3	
No significant findings	2	
Autolysis, moderate	1	
Skin	**2**	
No significant findings	2	
Small intestine	**9**	
Enteritis, mononuclear (lymphocytic, histiocytic)	2	
Enteritis, granulocytic (eosinophylic) by ciliates	1	
Protozoa, ciliates, no inflammation	1	
No significant findings	2	
Autolysis	5	
Spleen	**6**	
Hemosiderosis	3	
Splenitis, granulocytic (eosinophilic)	2	
Histiocytosis, red pulp	1	
White pulp hyperplasia	1	

Cont.

Stomach		**5**
	No significant findings	3
	Autolysis	2
Testicle		**6**
	Azoospermia	1
	Sexual maturity	1
	No significant findings	2
Tongue		**5**
	Degeneration, ballooning, Keratinocytes,	1
	Glossitis, mononuclear (lymphocytic)	1
	No significant findings	3
	Autolysis	1
Trachea		**8**
	No significant findings	8
Urinary bladder		**4**
	No significant findings	3
	Autolysis	1
Uterus		**1**
	No significant findings	1

Myocastor coypus (n=2)

Adrenal glands		**1**
	Autolysis	1
Brain		**1**
	Autolysis	1
Esophagus		**2**
	Adenitis, granulocytic (neutrophilic)	1
	No significant findings	1
Eye		**1**
	No significant findings	1
Heart		**2**
	Autolysis	2
Kidney		**2**
	Autolysis	2
Large intestine		**1**
	Autolysis	1
Liver		**2**
	Autolysis	2
Lungs		**2**
	Edema, alveolar	2
	Edema, perivascular	1
	Histiocytosis, alveolar	1
	Pneumonia, interstitial, granulocytic (neutrophilic), acute	1
Skeletal muscle		**2**
	No significant findings	1
	Autolysis	1
Small intestine		**1**
	Autolysis	1
Spleen		**2**
	Autolysis	2
Stomach		**1**
	Autolysis	1
Testicle		**1**
	Sexual maturity	1
Tongue		**1**
	No significant findings	1
Trachea		**1**
	No significant findings	1

		Cont.
Uterus		**1**
	No significant findings	1

***Rattus norvegicus* (n=1)**

Kidney		**1**
	Autolysis	1
Liver		**1**
	Autolysis	1
Pancreas		**1**
	Autolysis	1
Skeletal muscle		**1**
	Autolysis	1

***Guerlinguetus brasiliensis* (n=1)**

Adrenal glands		**1**
	Vacuolar degeneration, cortex, reticular	1
Esophagus		**1**
	No significant findings	1
Heart		**1**
	No significant findings	1
Kidney		**1**
	Mineralization, tubular, medullae	1
	Autolysis	1
Liver		**1**
	Autolysis	1
Lungs		**1**
	Edema, perivascular	1
	Hemorrhage, alveolar	1
Penis		**1**
	No significant findings	1
Prostate		**1**
	No significant findings	1
Small intestine		**1**
	No significant findings	1
Testicle		**1**
	Sexual maturity	1
Tongue		**1**
	No significant findings	1
Trachea		**1**
	No significant findings	1

APPENDIX X

Table 1. Host species screened for ticks

Tick-host associations	HI/HS	SL	O	Total number of ticks recovered				
				Larvae	**Nymphs**	**Males**	**Females**	**Total**
Amblyomma aureolatum								
Cerdocyon thous	2/49	PR, SP	4.1	0	0	2	1	3
Procyon cancrivorus	5/20	PR, SP	25	0	0	12	7	19
Puma concolor	1/4	SP	25	0	0	2	1	3
Amblyomma brasiliense								
Cerdocyon thous	2/49	MS, SP	4.1	0	12	0	0	12
Procyon cancrivorus	3/20	SP	15	0	10	0	0	10
Mazama gouazoubira	2/23	SP	8.7	0	4	0	0	4
Tamandua tetradactyla	5/41	SP	12.2	0	23	0	0	23
Amblyomma calcaratum								
Cerdocyon thous	1/49	MS	2	0	0	6	1	7
Tamandua tetradactyla	17/41	SC, SP	41.5	0	0	86	25	111
Amblyomma dubitatum								
Bradypus variegatus	1/5	SP	20	0	0	0	1	1
Didelphis aurita	3/14	SP	21.4	0	8	0	0	8
Hydrochoerus hydrochaeris	8/12	SP	66.7	0	182	94	48	324
Tamandua tetradactyla	1/41	SP	2.4	0	1	0	0	1
Amblyomma incisum								
Mazama gouazoubira	1/23	SP	4.3	0	10	0	0	10
Tamandua tetradactyla	1/41	SP	2.4	0	11	0	0	11
Amblyomma longirostre								
Coendou spinosus	6/7	SP	85.7	0	0	7	4	11

Amblyomma loricatus

Didelphis aurita	1/14	SP	7.1	0	0	1	0	1

Amblyomma nodosum

Cerdocyon thous	1/49	MS	2	0	0	2	0	2
Tamandua tetradactyla	6/41	SP	14.6	0	0	37	13	50

Amblyomma ovale

Cerdocyon thous	2/49	SP, SC	4.1	0	0	2	1	3
Chrysocyon brachyurus	1/5	SP	20	0	0	1	0	1
Galictis cuja	2/8	SP	25	0	0	0	3	3
Mazama gouazoubira	1/23	SP	4.3	0	0	6	1	7
Procyon cancrivorus	11/20	SP, SC	55	0	1	37	35	73
Puma concolor	3/4	SP	75	0	0	30	8	38

Amblyomma parkeri

Coendou spinosus	1/1	PR	100	0	0	1	0	1

Amblyomma sculptum

Cerdocyon thous	5/49	MS, SP	10.2	0	14	0	0	14
Chrysocyon brachyurus	2/5	SP	40	0	17	0	0	17
Dasypus novemcinctus	1/29	SP	3.4	0	1	0	0	1
Didelphis aurita	3/14	SP	21.4	0	41	0	0	41
Hydrochoerus hydrochaeris	7/12	SP	58.3	0	16	37	19	62
Mazama gouazoubira	2/23	SP	8.7	0	2	0	0	2
Procyon cancrivorus	1/20	SP	5	0	5	1	0	6
Tamandua tetradactyla	1/41	SP	2.4	0	0	0	1	1
Tapirus terrestris	2/2	MS	100	0	0	2	1	3

Amblyomma tigrinum

Chrysocyon brachyurus	1/5	SP	20	0	0	1	0	1

Amblyomma varium

Bradypus variegatus	4/5	SP	80	0	0	25	0	25
Tamandua tetradactyla	1/41	SP	2.4	0	2	0	0	2

Cont.

Amblyomma sp.

	HI/HS		O	PR	SP	MS	SC	
Cerdocyon thous	2/49	SP	4.1	10	0	0	0	10
Didelphis aurita	2/14	SP	14.3	48	0	0	0	48
Hydrochoerus hydrochaeris	2/12	SP	16.7	5	0	0	0	5
Leopardus pardalis	2/15	SP	13.3	5	0	0	0	5
Mazama gouazoubira	3/23	SP	13	17	0	0	0	17
Procyon cancrivorus	1/20	SP	5	2	0	0	0	2
Puma concolor	1/4	SP	24	42	0	0	0	42
Puma yagouaroundii	1/12	SP	8.3	11	0	0	0	11
Coendou spinosus	1/12	SP	8.3	2	0	0	0	2

Dermacentor nitens

Puma concolor	1/4	SP	25	0	0	2	0	2

Haemaphysalis juxtakochi

Mazama gouazoubira	11/23	SP	47.8	53	74	129	40	296

Ixodes aragoi

Mazama gouazoubira	6/23	SP	26.1	0	0	9	24	33

Ixodes loricatus

Didelphis aurita	3/14	SP	21.4	0	0	3	0	3

Ripicephalus microplus

Cerdocyon thous	1/49	SP	2	0	1	0	0	1
Dasypus novemcinctus	1/29	SP	3.4	0	2	0	0	2
Mazama gouazoubira	5/23	SP	21.7	5	12	30	34	81

Rhipicephalus sanguineus

Procyon cancrivorus	1/20	SP	5	0	0	1	0	1

HI=Hosts infested; HS=Hosts sampled; O=Occurrence; PR=Parana state; SP=Sao Paulo State; MS=Mato Grosso do Sul State; SC=Santa Catarina State