UNIVERSIDADE CATÓLICA DOM BOSCO
PROGRAMA DE PÓS-GRADUAÇÃO *STRICTO SENSU* EM
CIÊNCIAS AMBIENTAIS E SUSTENTABILIDADE AGROPECUÁRIA

Utilização da histopatologia como ferramenta para avaliação da saúde em uma população de *Holochilus chacarius* (rodentia:cricetidae) do Pantanal de Mato Grosso do Sul

Autora: Amanda Costa Rodrigues
Orientadora: Dra. Gisele Braziliano de Andrade
Co-orientador: Dr. Heitor Miraglia Herrera

CAMPO GRANDE/MS
Outubro, 2022

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UTILIZAÇÃO DA HISTOPATOLOGIA COMO FERRAMENTAPARA AVALIAÇÃO DA SAÚDE EM UMA POPULAÇÃO DE *HOLOCHILUS CHACARIUS* (RODENTIA:CRICETIDAE) DO PANTANAL DO MATO GROSSO DO SUL

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Campo Grande
Mato Grosso do Sul
Outubro, 2022

UNIVERSIDADE CATÓLICA DOM BOSCO
Inspira o futuro

Utilização da Histopatologia como Ferramenta para Avaliação da Saúde em População de *Holochilus chacarius* (Rodentia: Cricetidae) do Pantanal do Mato Grosso do Sul.

Autora: Amanda Costa Rodrigues
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A presente defesa foi realizada por webconferência. Eu, Gisele Braziliano de Andrade, como presidente da banca assinei a folha de aprovação com o consentimento de todos os membros, ainda na presença virtual destes.

Gisele B. de Andrade

Profa. Dra. Gisele Braziliano de Andrade (orientadora) – UCDB

Prof. Dr. Heitor Miraglia Herrera (coorientador) – UCDB

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Everyday, everyweek, every month and every year of my life, my hero's always 10 years away. I'm never gonna be my hero. I'm not gonna attain that. I know I'm not, and that's just fine with me because that keeps me with somebody to keep on chasing.

- Matthew McConaughey

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Dedico este trabalho ao meu avô, Adolvino Costa, por me mostrar o extraordinário mundo dos animais e me influenciar a me tornar uma médica veterinária.

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CEUA	Comitê de Ética no Uso de Animais
SISBIO	Sistema de Autorização e Informação em Biodiversidade
CC	Condição Corpórea
GLM	Generalized Linear Models

RESUMO

A população de pequenos mamíferos encontrada no Pantanal equivale a um terço de todos os mamíferos catalogados na área. Estes vem sendo considerados excelentes bioindicadores para o monitoramento da fauna e flora, pois são suscetíveis à mudança do habitat e podem desenvolver alterações fisiológicas e teciduais. O trabalho teve como objetivo utilizar a histopatologia como indicador de saúde de uma população de pequenos mamíferos do gênero *Holochilus*de uma fazenda de agro-ecoturismo no Pantanal de Mato Grosso do Sul, entre outubro e novembro de 2021. Informações sobre o animal, como peso, estado reprodutivo, sexo e comprimento cabeça-corpo foram fornecidas pelo grupo de pesquisa responsável pelas coletas, para posterior avaliação da condição corpórea. Fragmentos de pulmão, rim, pele do abdômen, fígado e sistema reprodutor conservados em formol, de 33 animais da espécie *Holochilus chacarius*, foram recebidos e submetidos ao processamento de histotecnia. As lâminas confeccionadas foram observadas ao microscópio óptico. As lesões encontradas foram classificadas em leves, moderadas e intensas. As alterações histopatológicas em fígado rins, pele e sistema reprodutor foram descritas como degenerativas e inflamatórias e variaram de leve a moderada, outrossim, nos pulmões, as alterações inflamatórias e a presença de pigmentos exógenos foram intensas em alguns animais. As alterações teciduais obtidas foram submetidas à análise estatística para relacionar as lesões com a condição corpórea destes animais. Observou-se que a maior intensidade dasalterações no pulmão desses animais foi a variável responsável por influenciar negativamente na condição corpórea dos indivíduos submetidos à análise. Conclui-se que a histopatologia é uma excelente ferramenta para monitoramento e avaliação da saúde dos pequenos mamíferos, podendo auxiliar como indicador de potenciais fatores de risco para os animais e para os humanos.

Palavras-chave: Bioindicador, histopatologia, condição corpórea

ABSTRACT

The population of small mammals found in the Pantanal is equivalent to one third of all mammals cataloged in the area. These have been considered excellent bioindicators for monitoring fauna and flora, as they are susceptible to habitat change and can develop physiological and tissue changes. The objective of this work was to use histopathology as an indicator of health in a population of small mammals of the genus *Holochilus* from an agro-ecotourism farm in the Pantanal of Mato Grosso do Sul, between October and November 2021. Information about the animal, such as weight, reproductive status, sex and head-body length were provided by the research group responsible for the collections, for later assessment of the body condition. Fragments of lung, kidney, skin of the abdomen, liver and reproductive system fixed in formalin, from 33 animals of the species *Holochiluschacarius*, were received and submitted to histotechnical processing. The prepared slides were observed under an optical microscope. The lesions found were classified as mild, moderate and severe. Histopathological changes in liver, kidneys, skin and reproductive system were described as degenerative and inflammatory and ranged from mild to moderate. In addition, in the lungs, inflammatory changes and the presence of exogenous pigments were intense in some animals. The tissue alterations obtained were submitted to statistical analysis to relate the lesions with the body condition of these animals. It was observed that the greater intensity of alterations in the lungs of these animals was the variable responsible for negatively influencing the body condition of the individuals submitted to the analysis. It is concluded that histopathology is an excellent tool for monitoring and evaluating the health of small mammals, and can help as an indicator of potential risk factors for animals and humans.

Keywords: bioindicator, body condition, histopathology

1. INTRODUÇÃO

O Pantanal é a maior planície alagável do mundo. É o habitat de uma vastadiversidadede animais, vegetações, solos e recursos hídricos, além das modificações impostas pelo homem, que ocupam o mesmo espaço, como as atividadesagropecuárias, rodovias e áreas de desmatamento (CATELLA, et al. 2010). Esta área possuium grande número de espécies de mamíferos catalogadas e estes animais são considerados excelentes biomarcadores (VIEIRA, 2014) pois podem desenvolver alterações fisiológicas e teciduais como resultado dessa intervenção humana (STERZ, et al. 2011).

Entretanto, justamente por ser um ecossistema diversificado, o Pantanal possui alta ocorrência de alguns agentes infecciosos e parasitários, devido à quantidade de vetores e o trânsito intenso de possíveis hospedeiros (SILVA, et al. 2004). Um problema sanitário notável do bioma é a presença de substâncias potencialmente tóxicas, capazes de poluir todo o ecossistema e sereminseridas na cadeia trófica de muitos animais. Essas substâncias exógenas podem ser oriundas do uso exagerado de agrodefensivos, resíduos de indústrias, minérios e potencialmente, das queimadas (CORINGA, 2014; ABREU & RIBEIRO, 2020). Todas essas interferências dentro do habitat podem causar estresse e desencadear doenças, interferindo na sanidade, fertilidade e desenvolvimento dos animais (LUIZ & ANTONIO, 2002).

Para medir o nível de interferência causado por esses fatores aos indivíduos, é possível utilizar biomarcadores, como os fisiológicos, moleculares, sanguíneos, histológicos e corporais que são essenciais tanto para avaliação do índice de saúde do animal, bem como da população e do sistema (MACEDA, 2015). A utilização da histopatologia como biomarcador das ações antrópicas vem ganhando cada vez mais espaço por conta da aplicação rápida e pela facilidade de detectar lesões em diversos órgãos de um só animal em apenas uma coleta, tornando-o ideal para estudo a campo (HILTON, et al. 1992). Nesse sentido, a pesquisa tem como objetivo verificar a eficácia da utilização do exame histopatológico como indicador de saúde individual de animais do gênero *Holochilus* no Pantanal de Mato Grosso do Sul. O trabalho foi realizado em parceriacomum grupo de pesquisa da Universidade Federal da Paraíba (UFPB), sendo parte de um projeto de doutorado.

2. OBJETIVOS

2.1 Objetivo geral

Avaliar o estado de saúde de *Holochilus chacarius* em uma área de agroecossistema do Pantanal de Mato Grosso do Sul.

2.2 objetivos específicos

- Identificar e quantificar as lesões teciduais encontradas em *Holochilus chacarius* em uma área de agroecossistema do Pantanal do MS;
- Relacionar a influência do quantitativo de lesões com a condição corpórea dos animais amostrados.

3. REVISÃO BIBLIOGRÁFICA

3.1 PEQUENOS MAMÍFEROS DO PANTANAL DO MATO GROSSO DO SUL

O bioma doPantanal ocupa uma área de 160.000km², porém levando-se em consideração as terras de países vizinhos, como o Paraguai e Bolívia, o Brasil tem cerca de 140.000km² do Pantanal (JUNK, et al. 2006). Conhecido mundialmente como um dos maiores ecossistemas alagados existente, o Pantanal possui uma grande diversidade de fauna e flora, em especial da mastofauna, devido às planícies abertas características da região (ARAUJO, 2001; RODELA & NETO, 2007). ANhecolândia é uma sub-região do Pantanal, na qual as inundações ocorrem de outubro a março, devido ao relevo do solo e seu clima é considerado subúmido. Tais inundações influenciam diretamente a diversidade da vegetação e consequentemente, na alimentação dos animais habitantes da região (RODELA, 2006; RODELA & NETO, 2007).

No bioma do Pantanal, ecossistema extensivamente ocupado pela pecuária e agricultura, avalia-se que existam entre 90 e 170 espécies de mamíferos catalogados, na qual pelo menos um terço é constituída pelos pequenos mamíferos, como marsupiais e roedores, de acordo com Layme, et al. (2014). Dentro da ordem dos marsupiais, pode ser avistada no Pantanal a família Didelphidae, que possui como membro as mais diversas espécies de cuíca, como a cuíca-lanosa-ocidental (*Caluromys lanatus*) e a cuíca-graciosa (*Gracilinanus agilis*). Na ordem Rodentia, várias famílias ocorrem no Pantanal, porém a mais abundante é a Cricetidae, que integra os popularmente conhecidos rato-do-mato (*Euryoryzomys nitidus* e *Cerradomys scotti*), rato-d’água (*Holochilus chacarius*) e rato-da-árvore (*Oecomys bicolor*) (ARAGONA, 2008).

Os marsupiais e roedores com o peso inferior a 1kg são denominados como pequenos mamíferos e assumem uma grande importância dentro do ecossistema em que habitam, pois são a base da cadeia alimentar de inúmeros grandes mamíferos, como felinos e canídeos (ABREU, et al. 2010; VIEIRA, 2014).

Em estudos realizados na região do Pantanal, sobre o uso do habitat e compartilhamento de espaço entre os pequenos mamíferos da fauna local, durante as capturas realizadas para identificação de indivíduos, podem ser observadas espécies das famílias como a Didelphidae, Cricetidae e Echimyidae (ANTUNES, 2009). As espécies catalogadas ocorreram tanto em áreas inundadas quanto em florestas, assim como em Campos limpos, Cerrados, Cerradões e baías de água salobra ou doce (ARAGONA, 2008). A frequência dos avistamentos e capturas dessas espécies de pequenos mamíferos podem variar de acordo com fatores ecológicos como fluxo de inundação e queimadas durante a estação seca, mas também por fatores causados intencionalmente, como o desmatamento e incêndios intencionais (COELHO, et al. 2010; RIBEIRO, et al. 2019). Além disso, a maior parte das espécies tende a se movimentar entre habitats em busca de terra seca, devido às variações climáticas típicas do Pantanal, entre estes animais estão os dos gêneros *Holochilus* e *Oecomys* (LÁZARI, 2011).

O gênero *Holochilus* pode ser dividido em quatro espécies, uma vez que apenas três delas podem ser observadas no Brasil: *H. brasiliensis*, descrito pela primeira vez em 1819, no sul do Brasil; *H. sciureus*, em 1842; *H. chacarius*, descrito em 1906; e por último, *H. lagigliai*, descrito apenas em 2015, ainda sem ocorrência em solo brasileiro (BRANDÃO & NASCIMENTO, 2015). Apesar de três das quatro espécies serem conhecidas há mais de um século, o gênero *Holochilus* ainda possui algumas características taxonômicas desconhecidas, assim como sua exata distribuição geográfica (PETERS, et al. 2013). Embora esse gênero possa ser frequente em todo o Brasil, o *H. chacarius*, em especial, ocupa o solo do Pantanal e inclusive áreas afetadas pelas queimadas (SEMEDO, 2022).

Os nomes populares associados ao gênero *Holochilus* diferem de acordo com a região do país. No sul do Brasil, é conhecido como “rato do junco” ou “rato da taquara”, já no Nordeste, como “rato da cana” (MARQUES, 1988). São roedores e possuem hábito semi-aquático. A princípio, são animais herbívoros que se alimentam de plantas terrestres e aquáticas, mas de acordo com o habitat e disponibilidade, podem incluir em sua dieta pequenos insetos, aranhas e moluscos. Tem hábitos diurnos em locais como cerrado e florestas do Pantanal (MARQUES, 1988; PARDIÑAS & TETA, 2011; KERBER, et al. 2012). De acordo com Lira, et al. (2015), a reprodução do gênero *Holochilus* é similar a dos outros pequenos mamíferos que convivem em clima tropical, com uma gestação de 21 dias e cerca de 5 dias de período fértil.

O *Holochilus chacarius* é um roedor com pelagem curta, membranas interdigitais, cauda igual ao comprimento da cabeça e do corpo e possui as patas traseiras ligeiramente mais palmadas. A cor do dorso é olivácea ou castanho-amarelada, gradualmente mais escura na linha média. (PATTON, et al. 2000). Sobre a distinção entre os sexos deste roedor, além do órgão genital, é possível reconhecer o sexo de acordo com o peso corporal, uma vez que os machos desse gênero podem pesar até 100g a mais do que as fêmeas, desde que estas não estejam no período de gestação (LIRA, et al. 2015).

Por serem animais que possuem membranas entre os dedos, o gênero *Holochilus* habita áreas com terra firme e água que também possuam oferta de comida e abrigo, tais como arrozais e plantações de cana-de-açúcar (MARQUES, 1988). Outro fator importante sobre o gênero *Holochilus* e outros pequenos mamíferos do Pantanal, é a capacidade de se tornar um reservatório de agentes parasitários, tais como *Schistosoma mansoni*, *Trypanosoma cruzi e* algumas espécies de filarídeos. Assim como podem ser portadores e auxiliar na transmissão de agentes infecciosos, que podem ser causadores de zoonoses, como aquelas da família *Flaviviridae* (BARRETTO & RIBEIRO, 1974; LIRA, et al. 2015; REZENDE, 2015).

3.2 ASPECTOS DAS CONDIÇÕES SANITÁRIAS E DE SAÚDE NO PANTANAL/MS

Alguns aspectos decorrentes da presença humana no Pantanal influenciam diretamente na sanidade dos animais de vida livre, aumentando as taxas de morbidade e mortalidade. Podem ser aquelas de origem geográfica ou ambiental, como os incêndios, contaminação das bacias hidrográficas, assoreamentos e desmatamento (SILVA, et al. 2008; PINTO, 2013). Outro fator diretamente ligado à presença de humanos na área é o risco de intoxicação por agrotóxicos e metais pesados. Os agrodefensivos são comumente utilizados para extermínio de pragas nas lavouras, mas podem afetar, por acidente, os animais silvestres da região, por meio da água, solo ou vegetação (SILVA, et al. 2004).

Em um estudo realizado sobre a presença de patógenos de caráter enzoótico em tatus de vida livre, Kluyber (2016) verificou que a ação antrópica no habitat desses animais pode desencadear uma alteração dos ciclos enzoóticos parasitários em decorrência da fragmentação do habitat natural, aumentando a disseminação de patógenos e o risco de ocorrências das zoonoses. Quando se trata daqueles agentes infecciosos transmitidos por vetores, como os flebotomíneos, triatomíneos e ixodídeos, as taxas de ocorrência das infecções podem ser alteradas drasticamente (JORGE, 2008; FIGUEIREDO, 2016).

3.2.1 Substâncias tóxicas

A quantidade de metais pesados liberados na natureza diariamente é um fato que causa preocupação às nações em geral, devido à contínua poluição do meio ambiente, dos alimentos produzidos na agricultura e pelo grande risco de intoxicação do ser humano e de animais (PIGNATI, et al. 2017). Considerado como um problema de saúde mundial foi anteriormente requisitado à Organização das Nações Unidas (ONU) que fossem elaboradas diretrizes para o uso consciente de químicos como agrodefensivos e fertilizantes, também instruindo punições adequadas para a utilização imprópria e prejudicial (RIGOTTO, et al. 2014; GOMES & SERRAGLIO, 2017) Estes agrodefensivos possuem constituintes declarados tóxicos e poluentes, como o Cádmio (Cd), Chumbo (Pb), Mercúrio (Hg), Cobre (Cu), Zinco (Zn), entre outros igualmente prejudiciais a sanidade animal e humana, quando há uso indiscriminado (GIMENO-GARCIA, et al. 1996).

Com os atuais estudos realizados para verificação do aumento desses metais pesados no meio ambiente, mensura-se que os elementos químicos mais presentes e também os mais nocivos aos seres vivos sejam o Chumbo e o Cádmio (CHIARI, et al. 2015). Tais contaminantes podem estar presentes em descartes de indústrias, na mineração, cartuchos de arma de fogo, baterias descartadas, metais, plástico, e pigmentos utilizados para preparação de tintas (CAPITANI, et al. 2009; TERÇARIOL, et al. 2010). A permanência em área de mineração também pode ocasionar o aparecimento dos sintomas da intoxicação (GUAGNINI, et al. 2018). O cultivo do arroz, em especial, tem sido empregada uma quantidade significativa de agrotóxicos devido ao aparecimento de roedores, considerados pragas desse cultivo (SANTOS, 2017; OLIVEIRA, 2020). Além da contaminação do próprio alimento, os químicos utilizados nos arrozais, tendem a ser transportados para as fontes de águas mais próximas, seja superficial ou subterrânea (SILVA, et al. 2009).

Diretamente relacionados à uma produção excessiva de radicais livres, os metais pesados induzem o organismo à uma defesa oxidativa prolongada, o que torna outras barreiras de defesa, como a hematoencefálica, cada vez mais vulneráveis. Após o dano na permeabilidade das barreiras antioxidantes, as moléculas conseguem chegar a todos os órgãos, inclusive ao sistema nervoso (MÉNDEZ-ARMENTA, et al. 2003; BRANCA, et al. 2020). As lesões individuais sistêmicas e seu impacto a nível populacional causados por metais pesados em pequenos mamíferos, em especial nos roedores, ainda não foram muito bem avaliados. Nota-se a diminuição no peso de órgãos como fígado e rins, consequentemente há diminuição do peso geral do animal (LEVENGOOD & HESKE, 2008). Isso pode decorrer devido a danos teciduais como atrofias, necrose e degenerações, seja vacuolar, hialina ou gordurosa. Outras lesões associadas aos metais pesados são: congestão, hemorragia, tumefação celular e respostas inflamatórias mononucleares e polimorfonucleares (DYK, et al. 2007; JIHEN, et al. 2008; BINKOWSKI, et al. 2013; UDOTONG, 2015; AKTER, et al. 2019).

É importante ressaltar que o Pantanal sul-matogrossense é uma área propícia às queimadas, devido à vegetação e seca prolongada, além da possibilidade de incêndio criminoso, geralmente por causa do impacto da pecuária na região (JUNIOR, et al. 2019). As queimadas liberam monóxido de carbono, composto altamente tóxico, incolor e inodoro, que pode levar a um quadro de intoxicação rápida e letal (INÁCIO & BRANDÃO, 2016),

3.2.2 Doenças infecto-parasitárias

Relacionadas diretamente à saúde pública do local, as doenças parasitárias são capazes também de trazer impacto econômico, além do impacto inicial na sanidade animal (FREITAS, 2017). Os parasitos podem afetar o habitat do animal hospedeiro de várias maneiras, seja alterando sua migração, sobrevivência, taxas de fecundidade esperada e até mesmo interferindo na cadeia alimentar, causando enfraquecimento do hospedeiro, por doença e redução de massa corporal ou por diminuição da densidade populacional (COMBES, 1995).

No Pantanal, a grande diversidade de animais conviventes em um mesmo ecossistema pode culminar na alta ocorrência de ectoparasitas, como os carrapatos, que por possuírem uma seletividade quase nula, podem servir como vetores de doenças entre mamíferos, aves, anfíbios, répteis e até humanos (WOLF, 2015). Outro vetor fortemente associado com a presença de doenças parasitárias são os flebotomíneos, que podem utilizar como reservatórios os pequenos mamíferos, como roedores e marsupiais (ACHILLES, 2018). Além disso, a transmissão de alguns patógenos pode ocorrer pelo contato com urina, fezes, saliva e pelos (SEIFOLLAHI, et al. 2016).

Portanto, devido à diversidade de fauna e vetores, os animais da região do Pantanal podem apresentar altas taxas de infecção parasitária, seja no trato gastrointestinal ou no sangue, tais como helmintos e protozoários (CATTO, 2000). Grande parte desses helmintos, por realizarem alguma parte de seu ciclo dentro do estômago ou intestino, tem seus os ovos eliminados pelas fezes, contaminando assim o habitat e consequentemente infectando outros animais que percorrem pelo mesmo solo e usam a mesma fonte de água (OVANDO & RIBEIRO, 2007; DIB & BARBOSA, 2020).

Os pequenos mamíferos, incluindo as espécies do gênero *Holochilus*, podem se infectar e permanecerem como hospedeiros de agentes como o *Schistosoma mansoni*, causador da esquistossomose, que pode ocasionar granulomas parasitários e lesões regressivas em órgãos importantes, como o pâncreas (ALCÂNTARA & DIAS, 1982; BASTOS, et al. 1985; GARCIA, 2017). O *Trypanosoma cruzi* e *Trypanosoma evansi* também são causadores frequentes de infecção em mamíferos de vida livre do cerrado brasileiro (HERRERA, et al. 2007). Responsável pela doença de Chagas, este protozoário pode afetar o tecido renal, cardíaco, pulmonar e nervoso, mostrando alterações como inflamação, hemorragia e a própria presença do protozoário (PEREIRA, 2017).

Os roedores também podem ser reservatórios de inúmeros patógenos enzoóticos, como a toxoplasmose, babesiose e a leishmaniose (SEIFOLLAHI, et al. 2016). A toxoplasmose é uma infecção considerada comum entre humanos, animais domésticos e silvestres de vida livre (FERRARONI & MARZOCHI, 1980). Em um estudo realizado no Pantanal de Mato Grosso, 11 onças-pintadas foram testadas para *Toxoplasma gondii* utilizando o teste de Reação de Imunofluorescência Indireta (RIFI) e verificou-se que 90,9% dos animais testados eram positivos para *T. gondii* (ONUMA, et al. 2016). O gênero *Leishmania*, causador de infecções endêmicas na região do Pantanal de Mato Grosso, e, a quantidade de reservatórios disponíveis na região, intensifica a transmissão (BRITO, 2019).

De acordo com uma pesquisa realizada por Mello (1981), para identificar infecções no sangue de roedores e marsupiais do Pantanal, além dos parasitos dentro do gênero *Trypanosoma*, foram encontradas microfilarias *Litomosoide scarini ie Grahamella* sp..O cerrado também tem um cenário propício para o crescimento das infecções causadas por agentes infecciosos como a *Rickettsia rickettsii*, devido ao número de vetores (CANÇADO, 2008). Além desses, os mamíferos podem entrar em contato e se tornarem hospedeiros de parasitos como: *Ancylostoma* sp, *Toxocara canis*, *Capillaria hepatica*, da família Taeniidae e outros de igual importânciazoonótica (BORGES, 2016).

3.3 INDICADORES DE SAÚDE ANIMAL

Os pequenos mamíferos podem atuar como bioindicadores de seu próprio habitat, auxiliando no diagnóstico sanitário do ambiente e na confirmação da presença de patógenos ou contaminantes (GARCÍA, et al. 2008). A avaliação da saúde desses mamíferos pode ser realizada por meio de análises como aferição da condição corpórea, densidade populacional, exame clínico, hemogramas e perfil bioquímico, até testes mais complexos como moleculares, sorológicos e toxicológicos, dependendo sempre do fator prejudicial envolvido (WIDMER, 2009; CHAMBO, 2011). No caso de fatores que desencadeiam lesões teciduais, pode-se optar pela realização de um exame histopatológico, para estudo dos danos microscópicos ocasionados por tal agente. O exame histopatológico é um biomarcador histológico capaz de auxiliar nos casos de poluição ambiental e bioacumulação de substâncias, pois mostra alterações como morte celular, degenerações e respostas inflamatórias (SILVA, 2015; DAMASIO, 2017).

Além de serem excelentes bioindicadores, os pequenos mamíferos contaminados ou portadores de alguma doença podem incorporar tal complicação na cadeia alimentar, justamente por ser um grupo que serve de base na cadeia trófica de muitos animais da região (COSTA, 2020). No caso dos metais pesados, por exemplo, devido à característica acumulativa dessas substâncias, seu predador e o resto da cadeia também serão comprometidos (ANDRÉA, 2008)

Os animais de vida livres são utilizados como bioindicadores da saúde do ecossistema em que vivem e das alterações sofridas pela fauna, principalmente aquelas causadas pela presença do homem (LOBATO, 2012; SILVA, et al. 2016). Porém, são escassas as pesquisas que abordem infecções parasitárias (MARQUES, 2019), intoxicação (PEREIRA, 2010), atropelamentos (RAMOS-ABRANTES, et al. 2018; SANTOS, 2021), tráfico (CUNHA, 2016) e tudo mais que possa afetar os animais silvestres a nível de saúde do indivíduo. Alguns parâmetros utilizados como indicadores de saúde dentro da medicina veterinária legal, geralmente voltada para os animais domésticos, também são viáveis para serem utilizados em animais silvestres sem interferência invasiva, como: observar a locomoção, estado corporal, presença de ectoparasitas e/ou de lesões macroscópicas externas, áreas em que o animal circula e aparência das fezes (GARCIA, et al.2019).

O monitoramento das alterações causadas por fatores estressantes no ecossistema em que o animal está inserido pode servir como um indicador de saúde. Uma forma de classificar esses marcadores é separá-los em histológicos ou fisiológicos, principalmente no monitoramento da saúde de algumas espécies de peixes (WINKALER, et al. 2001). Dentro da categoria fisiológica, a variação do peso do animal, além de indicar a presença de algum estresse, pode ser uma reação causada por desequilíbrios de outros marcadores, como os sanguíneos, moleculares e bioquímicos (FREIRE, et al. 2008; OLIVEIRA, et al. 2010).

As alterações teciduais microscópicas identificadas e medidas por meio do exame histopatológico são uma ótima opção para avaliar o nível de saúde dos animais e do ambiente (OLIVEIRA, et al. 2016). Além disso, os biomarcadores histopatológicos já são vastamente utilizados na constatação de tumores (COSTANTINI, et al. 2015). A utilização de cada órgão vai ser determinada de acordo com o risco ao qual o animal está exposto. O fígado e rim, por exemplo, são excelentes biomarcadores nos casos de intoxicação (ALBINATI, et al. 2009), uma vez que as lesões causadas por tóxicos são maiores em órgãos de metabolização. A histopatologia vem sendo utilizada como biomarcador em muitos estudos devido à facilidade de coleta das amostras, assim como a capacidade de poder analisar vários órgãos de um só indivíduo e da possibilidade de identificar lesões específicas e diferenciar alterações crônicas e agudas (AMEUR, et al. 2012).

A condição corpórea é um indicador fisiológico, dado que o peso pode declinar devido a fatores estressantes do ecossistema, como por exemplo, a presença de poluentes (RAJKUMAR, 2008). Em estudo realizado por Levengood e Heske (2008), foi constatado que o peso corporal de animais residentes em um ambiente contaminado por metais pesados diminuiu, conforme a exposição do poluente aumentou, embora não tenha sido constatada uma diferença grande. Animais com peso corporal ideal demonstram melhor desempenho reprodutivo e de saúde geral (MACHADO, et al. 2008), enquanto aqueles que foram afetados por algum fator e consequentemente têm uma piora no índice corporal, serão os mais suscetíveis a coinfecções. Caso o animal também mostre um quadro de anemia, as chances de infecções podem ser quadruplicadas (BELDOMENICO & BEGON, 2009). O parasitismo também é um fator que compromete a massa corporal em decorrência da falta de apetite e comprometimento no crescimento animal (IRVINE, et al. 2006). Portanto, a verificação da condição corpórea do animal estudado auxilia a monitorar a resposta do indivíduo às mudanças do ambiente, inclusive daqueles que habitam locais com fortes interferências humanas (CREMONESI, et al. 2021).

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ARITGO 1

Use of histopathology as a tool for health assessment in a population of *Holochilus chacarius* (rodentia:cricetidae) from the Pantanal of Mato Grosso do Sul

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HIGHLITGH

The animals were experienced high exposure to rural fires.

The use of the histopathological matrix was efficient to evaluate the intensity of tissue alterations.

The analysis of histopathological changes can be used as a tool for health assessment.

Animals with the highest number of lung lesions had worse body condition.

ABSTRACT

Small mammals can be used as bioindicators because they have a short life span and are strictly associated with the environment in which they live, quickly responding to the consequences of anthropic changes. The objective of this work was to use histopathology to evaluate the health of a population of *Holochilus chacarius* captured near a rice crop inside an agroecotourism farm in the Pantanal of Mato Grosso do Sul, during the months of October and November 2021. At the time of removal in the field, measurements of weight and length were obtained in order to assess the body condition of the animals. Fragments of lung, kidney, skin of the abdomen, liver and reproductive system of 33 animals were submitted to histotechnical processing and the slides were observed under an optical microscope. The tissue injuries observed were classified as mild, moderate and severe. The histopathological analyzes were quantified and tabulated forming a matrix for further statistical analysis. We used Generalized Linear Models to verify if there was any influence of tissue changes on the body condition. We observed degenerative and inflammatory changes in the liver, kidneys, skin and reproductive system that ranged from mild to moderate. In the lungs, we found that the inflammatory changes and the presence of exogenous pigments were severe in some animals. It was observed that the intensity of the lesions found in the lungs had a negative influence on the body condition. The histopathological lesions observed in this study may be associated with anthropic action, such as the presence of pneumoconiosis and lesions suggestive of exposure to heavy metals, such as those seen in the lung, kidney and liver. The present study includes ecopathology in the single health approach by helping to understand the relationships that occur in the tissue environment.

Keywords: bioindicator, body condition, histopathology, small mammals

1. Introduction

Free-living wild animals are constantly exposed to imbalances imposed on the environment they inhabit and, consequently, to the health issues triggered by them (Silva et al., 2010). In the Pantanal, it is still possible to add up the presence of a huge diversity of animal species, including insect vectors of infectious-parasitic agents (Wolf, 2015; Rocha and Santos., 2016).

From the study of a species or group of animals in a given habitat, it is possible to measure the quality of the ecosystem, environmental impacts and the presence of factors such as the use of toxic substances, deforestation, burning gases and other impacts (Pretes and Vincenci, 2019). According to the One Health concept, these factors do not affect only the animal and the environment, but also the human being, showing that the balance within the ecosystem is important for the evolution and control of emerging diseases (Destoumieux-Garzón et al., 2018). Added to this concept, ecopathology is a branch of pathology aimed at understanding the risk factors in a given environment and their relationship with the emergence of diseases (Silva et al., 1998). The so-called animal bioindicators have also been widely used as indicators of areas that are constantly being interfered by human presence, such as in places with agricultural activity (Rocha et al., 2005; Oliveira et al., 2014).

In order to perform the analysis and individual susceptibility profile of each animal, biomarkers can be used (Amorim, 2013). The use of these indicators depends exclusively on the type of contamination the animals are exposed, among which are available for application: histological, toxicological, genetic, hematological, enzymatic, immune function and body condition (Martins, 2010; Leotta, et al., 2012; Cabrera, 2013).

Histopathological analysis is an effective and fast method for detecting biological effects such as chronic and acute injuries that can be caused by any environmental contamination (Liebel et al., 2013). It is also possible to assess tissue damage resulting from parasitic infections, as in the case of helminths, commonly found in free-ranging animals (Oyarzún-Ruiz et al., 2019). The histological marker can be used in field and laboratory studies, since it presents ease of collection and the possibility of evaluating several organs of the same individual (Ameur et al., 2012). The histopathological examination is effective in verifying the health of the animal as an individual, as it shows changes such as cell death, degenerations and inflammatory responses (SIlva, 2015).

In the present work, we aimed to investigate whether the health status of a population of *Holochilus chacarius* captured on an agroecotourism farm in the Pantanal de Miranda/MS and exposed to anthropic actions would be influencing the presence of the histopathological changes observed.

2. Materials and method

The collection of biological material was conducted at the agroecotourism farm near Miranda, in the Pantanal of Mato Grosso do Sul, between October and November 2021, as part of the doctoral work of the student Érica Fernanda Gonçalves Gomes de Sá, UFPB. The procedures performed during this research are in accordance with licenses CEUA-PB nº 9192091019, CEUA UCDB 006/2022 and SISBIO 73472. The capture of wild animals was authorized by the Capture License ICMBio/MMA nº 72681. Tissues from 33 *Holochilus chacarius* were sent for histopathological examination at Dom Bosco Catholic University, 21 males and 12 females. The samples were individually fixed with 10% buffered formalin. Also, information about sex, reproductive status, weight and head-body length were recorded. The area was strongly affected by the fires that occurred in 2021 (Trindade, 2021). Furthermore, a previous study demonstrated circulating levels of Lead, Cadmium, Iron and Zinc in the small mammals that inhabit the area (from Sá É.F.G.G. Personal communication).

2.1 Histopathological examination

For the histopathological examination, fragments of lung, liver, kidneys, skin of the abdomen and reproductive system were selected. The fixed tissues were cleaved into 3 mm to 5 mm fragments and sent to the Histopathological Technical Center of Curitiba for preparation and staining of slides with Hematoxylin and Eosin (HE); Periodic Acid-Schiff (PAS), for renal protein; Prussian Blue, for visualization of ferric pigments; Congo red for renal amyloidosis; and Masson's Trichrome for connective tissue. The histological slides were analyzed using the light composite light microscope

Carl Zeiss Microscopy GmbH (MOC) model Axio Scope A1 and captured with the aid of Zen software. Tissue images were photographed by an Axiocam 503 color camera attached to the MOC.

Each tissue was individually analyzed and the changes were classified as regressive, circulatory, and inflammatory and repair (Binkowvski, 2013). The intensity of changes was classified as absent (0), mild (focal-1), moderate (multifocal to coalescing-2) or severe (extensive-3). The histopathological observations were tabulated forming a matrix adapted from Leopoldo (1971) for further analysis, thus obtaining a score for each animal as a result of the amount and intensity of changes recorded.

2.2 Statistical analysis

Generalized Linear Models (GLM) were created to explore the effects of lesions in the lung, liver, skin, kidney and reproductive system on the Body Condition (BC) of *H. chacarius*. In addition, a complete model was created using all variables (all lesions), and a null model. BC was calculated using the residual of an ordinary linear regression between body weight (g) and head-body length (mm). In this study, BC was used as a health assessment parameter (Santos et al., 2018; 2022). All candidate models were compared in a model selection approach based on the Akaike information criterion corrected for small samples (AICc – Akaike Information Criterion corrected) (Akaike, 1974), considering all models with $\Delta AICc \leq 2$ plausible, the analyzes were performed using the 'AICcmodavg' package version 2.3–1 (Mazerolle, 2020) in R 3.5.0 (Team R Development Nucleo, 2018).

3. Results

Different types of pathological alterations were observed, which varied in terms of intensity from mild to moderate, with the exception of the lung, in which some alterations were intense. Inflammation, degeneration and congestion were found in the liver, lung, kidneys and reproductive system. In the skin we observed only inflammation that ranged from medium to moderate. We draw attention to the exogenous particles found in the lung and liver.

The pathological changes recorded for each tissue, along with the different intensities observed, are presented in Table 1. Combining all the changes and scores, an individual could have a maximum score of 78. Overall, the results showed that the maximum value obtained was of 28 (n=2) (Table 1).

ID	Liver						Skin		Lung								Reproductive							Kidney								OVERALL
	Circulatory Alteration	Inflammatory and Repair Alteration		Regressive Alteration			Inflammatory and Repair Alteration		Circulatory Alteration		Inflammatory and Repair Alteration		Regressive Alteration				Circulatory Alteration	Inflammatory and Repair Alteration	Regressive Alteration					Circulatory Alteration		Inflammatory and Repair Alteration	Regressive Alteration					
	Congestion	Fibrosis	Inflammation	Degeneration	Exogenous Particles	Total	Inflammation	Total	Congestion	Hemorrhage	Atelectasis	Inflammation	Degeneration	Emphysema	Exogenous Particles	Total	Congestion	Inflammation	Absence of Sperm	Degeneration	Necrose	Pigments	Total	Congestion	Hemorrhage	Inflammation	Glomerular atrophy	Degeneration	Glomerular Hypertrophy	Protein Accumulation	Total	
EGS 37	2	1	0	0	0	3	1	1	3	0	1	2	0	2	2	10	1	0	1	1	0	0	3	3	0	1	2	2	2	1	11	28
EGS 76	1	0	1	0	0	2	1	1	3	1	1	2	0	2	1	10	0	0	2	2	0	0	4	2	0	0	2	3	2	2	11	28
EGS 38	1	0	0	0	0	1	0	0	3	0	3	3	0	3	2	14	2	0	1	0	0	0	3	0	2	0	2	0	3	1	8	26
EGS 64	2	0	0	2	2	6	0	0	1	0	3	3	0	3	2	12	0	0	0	1	0	0	1	2	0	0	2	1	0	2	7	26
EGS 30	2	1	1	0	0	4	0	0	2	0	2	2	0	2	2	10	2	0	2	0	0	0	4	3	0	0	1	0	2	1	7	25
EGS 51	2	1	1	0	2	6	0	0	1	0	2	1	0	0	2	6	0	0	2	2	0	0	4	1	1	0	2	1	2	1	8	24
EGS 31	0	0	0	0	0	0	0	0	3	2	2	2	0	3	1	13	3	0	0	0	0	2	5	2	0	0	1	0	0	2	5	23
EGS 33	1	0	0	0	1	2	0	0	1	0	2	2	0	2	3	10	1	0	2	2	0	0	5	2	0	0	0	1	2	1	6	23
EGS 72	0	0	1	0	1	2	1	1	0	1	1	2	0	0	1	5	1	0	1	2	0	0	4	2	0	2	2	3	2	0	11	23
EGS 39	1	0	0	0	0	1	1	1	3	0	3	0	0	3	0	9	1	0	2	0	0	0	3	2	0	0	0	2	2	2	8	22
EGS 71	0	1	1	0	0	2	2	2	2	0	1	2	0	1	1	7	0	0	0	2	0	0	2	2	0	1	1	2	2	1	9	22
EGS 98	1	0	1	1	0	3	0	0	1	3	0	1	0	1	1	7	0	0	0	1	0	0	1	1	1	0	3	1	2	2	10	21
EGS 27	2	0	0	0	1	3	0	0	2	0	1	1	0	1	2	7	0	0	2	1	0	0	3	2	0	0	1	1	2	1	7	20
EGS 34	2	0	2	0	0	4	1	1	0	0	1	2	0	2	1	6	0	0	0	0	0	1	1	2	0	1	2	0	2	1	8	20
EGS 50	1	0	0	3	2	6	2	2	0	0	1	2	0	1	2	6	0	0	0	1	0	0	1	0	1	0	2	0	0	2	5	20
EGS 61	0	0	2	2	0	4	1	1	2	1	1	2	0	3	1	10	0	0	0	1	0	0	1	0	0	0	1	1	2	0	4	20
EGS 22	3	1	0	0	0	4	0	0	2	0	2	3	0	3	2	12	1	1	0	0	0	0	2	0	0	0	1	0	0	0	1	19
EGS 32	1	0	0	2	0	3	0	0	1	0	1	2	0	2	0	6	0	1	2	0	0	0	3	3	0	0	1	1	2	0	7	19
EGS 36	1	0	0	0	0	1	0	0	0	0	2	2	0	3	2	9	0	0	0	1	0	0	1	2	1	0	1	2	2	0	8	19
EGS 41	2	0	0	0	0	2	0	0	0	2	2	0	1	0	1	6	0	0	1	1	0	0	2	3	1	0	2	0	2	1	9	19
EGS 57	2	0	0	0	0	2	1	1	0	0	1	2	0	1	2	6	0	0	2	1	0	0	3	1	0	2	0	1	0	2	6	18
EGS 59	1	0	0	1	1	3	1	1	2	0	1	2	0	2	2	9	0	0	0	0	0	0	0	1	0	0	2	1	1	0	5	18
EGS 60	3	0	1	0	2	6	1	1	1	0	1	1	0	2	0	5	0	0	0	0	0	0	0	0	1	1	2	1	0	1	6	18
EGS 23	1	0	0	0	1	2	1	1	0	0	0	2	0	2	2	6	2	0	2	0	0	0	4	0	0	0	0	0	2	1	3	16
EGS 40	0	0	1	1	0	2	1	1	0	0	0	2	0	3	1	6	0	0	2	1	0	0	3	0	0	1	1	0	1	1	4	16
EGS 58	1	0	0	0	1	2	1	1	2	0	1	2	1	2	1	9	0	0	1	0	0	0	1	0	0	0	0	0	1	2	3	16
EGS 62	0	0	0	1	0	1	0	0	2	1	0	2	0	2	1	8	0	0	1	1	0	0	2	1	0	0	0	1	2	1	5	16
EGS 63	2	0	2	0	0	4	0	0	2	0	1	2	0	0	1	6	1	0	0	0	0	0	1	0	1	2	0	1	0	1	5	16
EGS 25	2	0	1	0	1	4	0	0	1	0	1	2	0	3	1	8	0	0	0	0	0	0	0	0	0	0	0	0	2	1	3	15
EGS 48	0	0	0	2	0	2	0	0	2	0	2	1	0	3	0	8	0	0	0	0	0	0	0	0	0	0	2	0	3	0	5	15
EGS 42	1	0	0	0	0	1	1	1	1	0	0	0	0	0	1	2	0	1	0	2	0	0	3	0	0	2	0	0	1	1	4	11
EGS 49	2	0	0	0	1	3	2	2	0	0	0	1	0	1	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	1	2	10
EGS 65	0	0	0	1	0	1	0	0	0	0	1	0	0	3	1	5	0	0	0	0	0	0	0	0	1	1	0	2	0	0	4	10
																																78

Table 1: Histopathological matrix showing the different intensities of the alterations found in different tissues of Holochilus chacarius sampled in the Pantanal de Miranda, Mato Grosso do Sul, between October and November 2021. (0) absent, (1) mild, (2) moderate and (3) intense.

In the lungs, we observed seven different lesions, although atelectasis, congestion, emphysema and pigments were the ones that occurred with greater intensity (intense-3) (Table 1). Furthermore, we detected that 28 animals (84.8%) had pneumoconiosis, accumulation of exogenous anthracosis-like particles (Figures 1A and 1B). The alveolar sacs were full of mixed inflammatory infiltrate and red blood cells, in addition to thickening of the alveolar septa, characterizing an obstructive atelectasis ranging from moderate to severe observed in 81.8% of the studied individuals (Fig. 1A). Around these areas, areas of compensatory pulmonary emphysema were observed (Fig. 1B). In the lungs of three animals, microfilariae were found inside the vessels and bronchi (Fig. 1C).

Renal tubules and glomerular space showed accumulation of protein (75.7%), later confirmed by Periodic Acid-Schiff (PAS) staining (Fig. 1D). The kidneys showed regressive changes, generally classified as mild to moderate, such as degeneration of renal tubule epithelial cells in 57.5% of the sampled animals (Fig. 2A). Glomerular atrophy was also observed in 69.7% of the animals and glomerular hypertrophy in 72.7% of the evaluated individuals (Fig. 2B), resulting in a decrease of the Bowman's space. Furthermore, we observed multifocal areas of hemorrhage and congestion in the renal parenchyma.

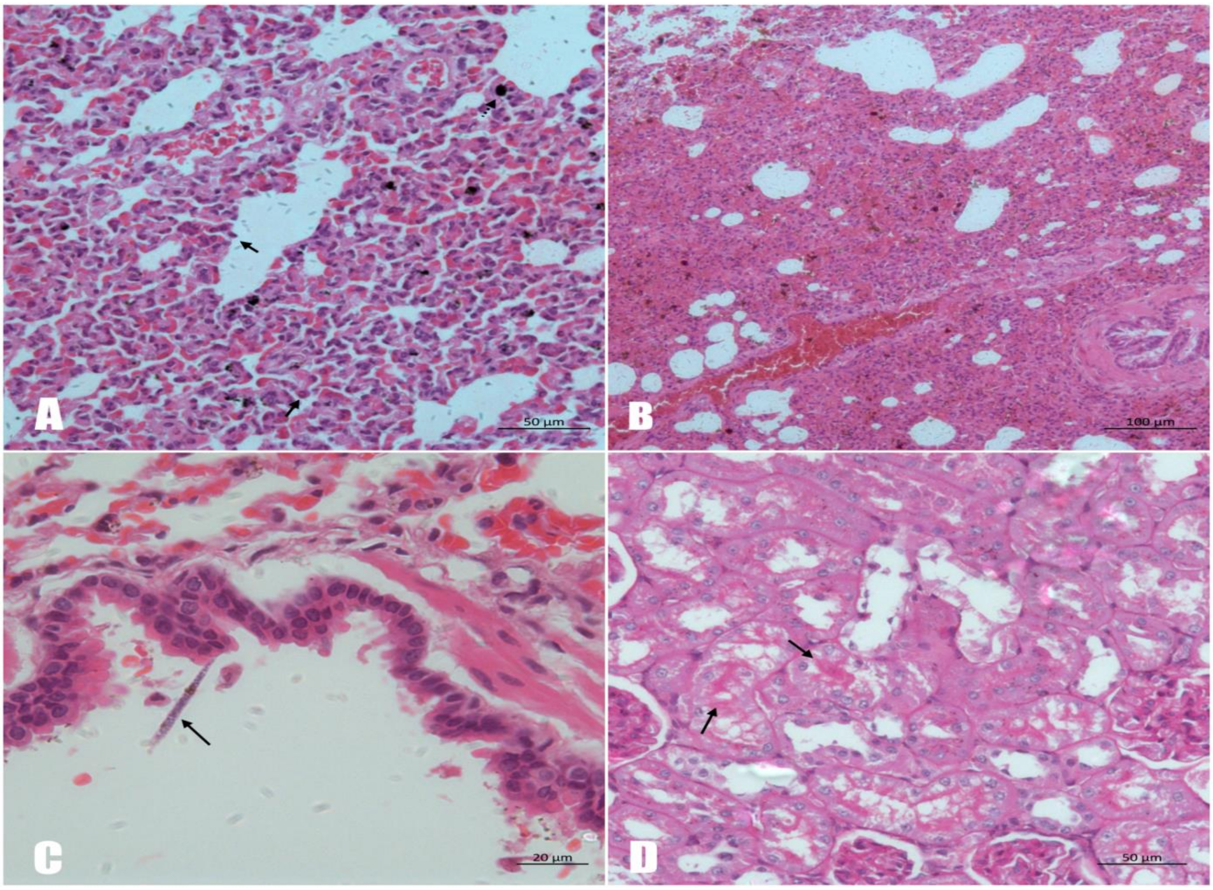

Fig. 1 – Lung and Kidney of *Holochilus chacarius*. (A) Lung - Dark colored particles (full arrow), inflammatory cells and red blood cells in the alveolar space. (B) Lung showing atelectasis and emphysema. (C) Microfilaria within the lumen of a bronchus in a lung (arrow); (D) Kidney showing protein accumulation, detected by PAS staining.

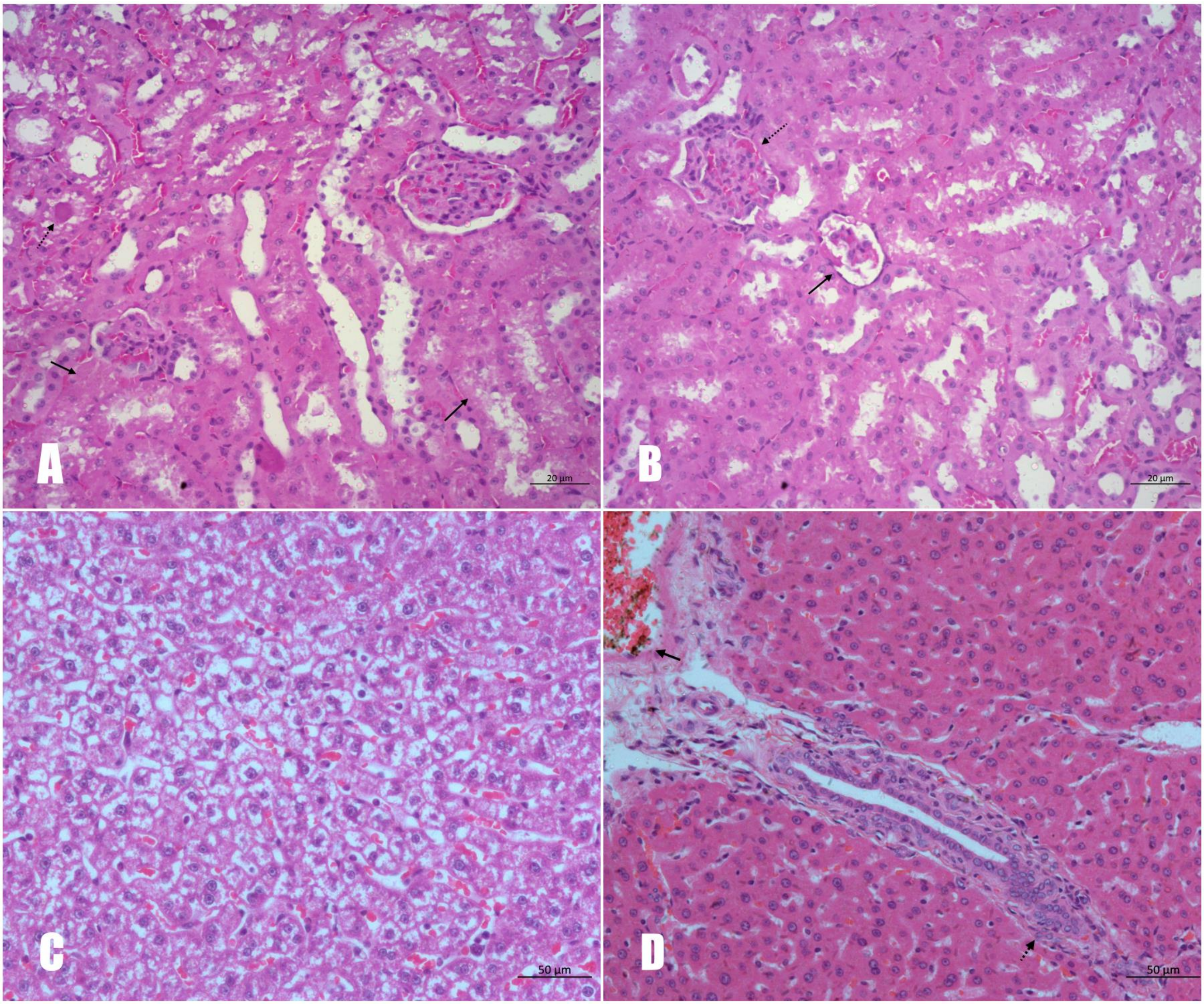

Fig. 2 – Kidney and liver of *Holochilus chacarius*. (A) Vacuolization of renal tubule epithelial cells (arrows) and presence of protein (dotted arrow). (B) Atrophied renal glomerulus (full arrow) and hypertrophied glomerulus, with absence of Bowman's space. (C) Liver showing hydropic degeneration of hepatocytes; (D) portal spaces with proliferation of bile duct epithelial cells (dotted arrow) and dark colored pigments (full arrow).

In the liver, circulatory disorders, mainly congestion, were classified as mild to moderate. Hydropic degeneration of hepatocytes was observed in nine individuals (27.3%), manifested by intense intracytoplasmic vacuolization (Fig. 2C). The presence of blackened pigments inside and outside the blood vessels, similar to those found in the lung, was visualized. Only five animals (15%) presented portal spaces with proliferation of bile duct epithelial cells (Fig. 2D), of which two were severely parasitized by the helminth of the genus *Capillaria*, showing tissue migration and egg deposition in the hepatic parenchyma surrounded by inflammatory cells (Fig. 3A and 3B). No significant changes were noticed in the skin. Few individuals had nonspecific inflammation, of mild to moderate intensity. In this particular tissue, the areas of inflammation were focal, with few polymorphonuclear cells in connective tissue from 15 subjects (45.5%).

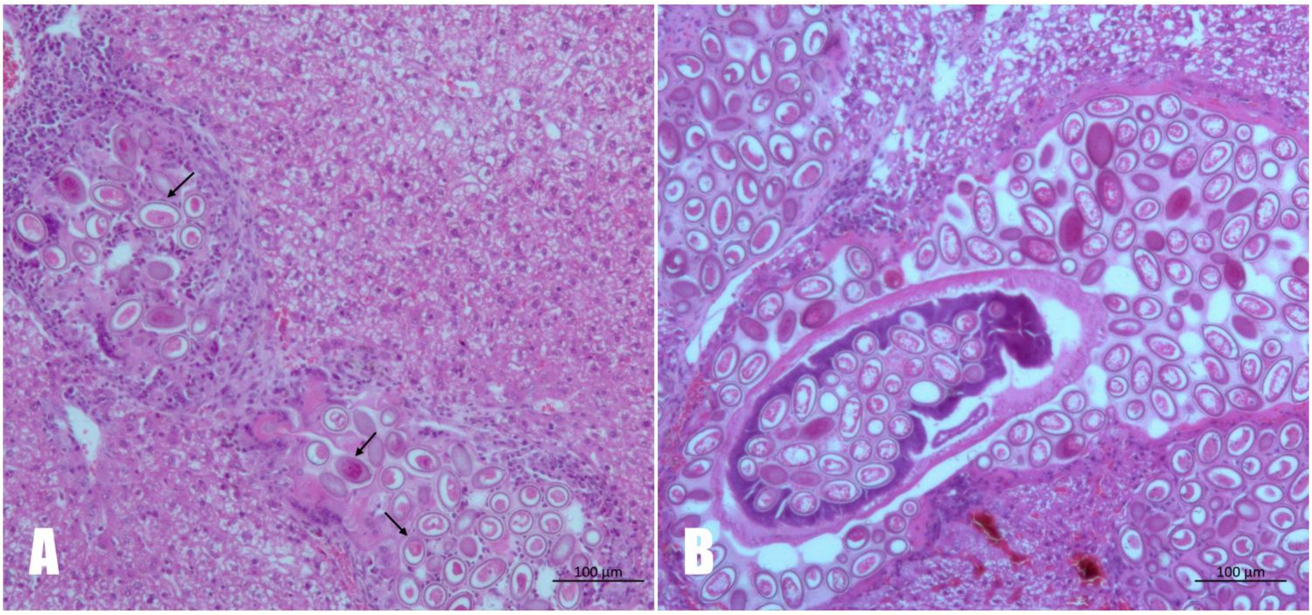

Fig. 3 – Liver of *Holochilus chacarius.* (A) Parasitic reaction, containing eggs of nematodes of the genus *Capillaria* deposited in the liver parenchyma (arrow) and mixed inflammatory reaction around it; (B) Liver containing adult parasite of the genus *Capillaria*, with eggs inside.

The male reproductive system was evaluated from the observation of the testis and epididymis. Both tissues showed foci of degeneration of the seminiferous tubules and epididymal ducts in 18 animals (54%), ranging from mild to moderate, associated with the presence of multinucleated giant cells observed in only two animals. The absence of sperm in these two structures, even in scrotaled animals, was considered a regressive change (Fig. 4A) observed in 15 animals (45.5%). In the reproductive system of females, few tissue alterations were found, such as the presence of the pigment lipofuchsin in one individual (Fig. 4B).

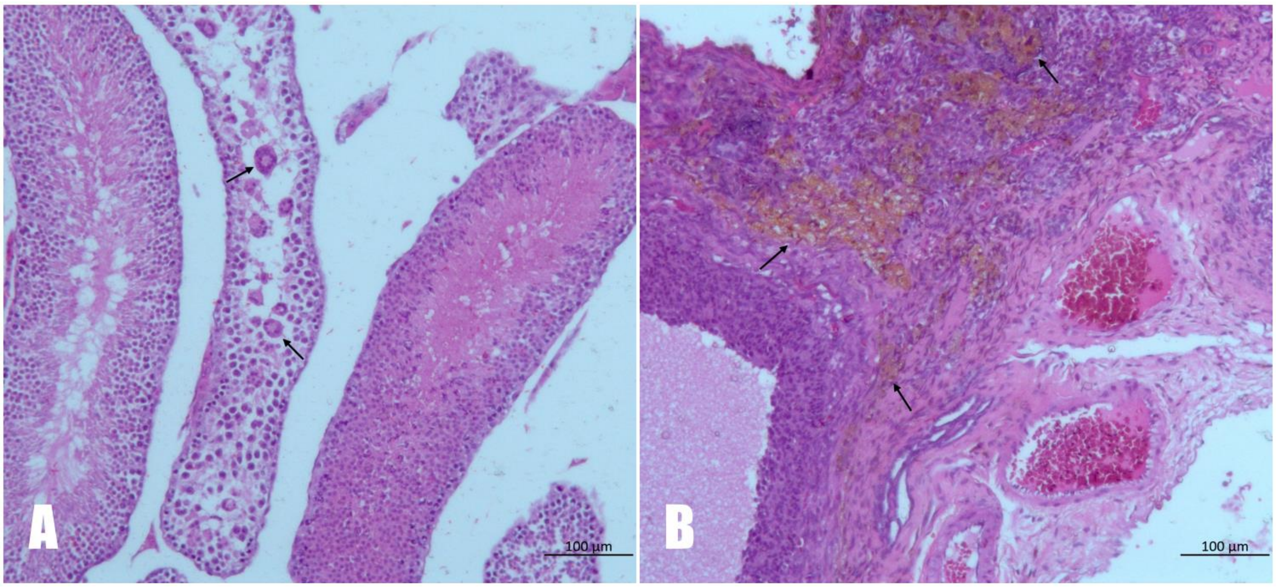

Fig. 4 – **Reproductive system of *Holochilus chacarius***. (A) Seminiferous tubules of a scrotal male, showing degeneration with disorganized germ cells and multinucleated giant cells (arrows). Note tubules without lumen and absence of sperm; (B) Ovary of female in the reproductive period, showing lipofuccin pigment in tissue.

The presence of ferric pigment, amyloids and connective tissue were not observed through the special stains (Prussian Blue, Congo Red and Masson's Trichrome).

GLM analyzes showed that the BC of *H. chacarius* was negatively influenced by lung lesions (ΔAICc: 0; AICcWt: 0.70; 95% CI = -3.67 to -0.44, p-value = 0.01). That is, individuals with greater intensity of pulmonary lesions have lower BC (Table 2).

Table 2. Models used to explore the variables that influenced the Body Condition of *Holochilus chacarius* captured in the Pantanal, Miranda-MS, K=Number of Parameters; AICc=Akaike Information Criterion; ΔAICc=Delta Akaike Information Criterion; AICcWt=Akaike's weight; Cum.Wt=Accumulated Akaike Weight.

Modelos	**K**	**AICc**	**ΔAICc**	**AICcWt**	**Cum.Wt**
Model 2 (body condition ~ lung injuries)	3	276,79	0,00	0,70	0,70
Null Model (body condition ~ 1)	2	280,40	3,61	0,12	0,82
Model 5 (body condition ~ liver injuries)	3	281,99	5,20	0,05	0,87
Model 4 (body condition ~ kidney injuries)	3	282,14	5,35	0,05	0,92
Model 3 (body condition ~ skin injuries)	3	282,74	5,95	0,04	0,95
Model 1 (body condition ~ reproductive injuries)	3	282,80	6,01	0,03	0,99
Model 6 (body condition ~ all injuries)	7	285,25	8,46	0,01	1,00

4. Discussion

In the present study, lung lesions were the most significant to assess health, reflecting negatively on the body condition of the animals analyzed. In fact, it has been found that the toxicity caused by heavy metals in vital organs such as the lung, especially when inhaled, can cause a decrease in body condition (Pandey and Madhuri, 2016). As there is a history of circulating levels of Lead, Cadmium, Iron and Zinc in the small mammals that inhabit the sampled area (from Sá É.F.G.G. Personal communication), probably the severe regressive lesions such as emphysema, degeneration and pigments and the inflammatory lesion such as atelectasis observed in the investigated animals would result from exposure to these contaminants. It has been shown that regressive pathological changes, such as emphysema, are directly linked to a reduction in the body condition of animals

(Tseyage et al., 2016; Rich et al., 2020), since prolonged exposure or inhalation to toxic substances causes a progressive lung disease, negatively interfering with food consumption (Fehrenbach, 2006). Furthermore, the presence of pulmonary emphysema causes dyspnea, which activates the sympathetic nervous system by increasing the release of catecholamines, which causes increased energy consumption as a result of hypermetabolism (Hofford et al., 1990). Also, the anthracosis observed in 28 of the 33 animals studied may be strongly associated with the soot released into the environment as a result of the fires that occurred in 2021 in the Pantanal Sul Mato Grossense region (Trindade, 2021). of the environmental condition by inhalation of carbon monoxide, due to the rapid onset of clinical symptoms and influence on the decrease in body mass (Mutluoğlu et al., 2016). On the other hand, a study carried out in 2003 with birds that died in a with relevant levels of cadmium and lead in the environment, showed that these animals presented anthracosis on histopathological examination and still had excellent or good body condition (Defranceschi et al., 2003). Loss of physical condition in free-ranging animals can be associated with other factors such as gastroenteritis, abscesses, trauma and pneumonia caused by the presence of parasites (Aguirre et al., 1999).

In the kidney, tissue lesions of regressive, inflammatory and circulatory origin were observed, which may be associated with three mechanisms of action of some toxic substances: (i) oxidative stress, which increases the resistance of the vascular system and increases blood pressure; (ii) by increasing free radicals in the body, causing inflammation; and (iii) enzymatic alterations and apoptosis that culminate in degenerative and necrotic alterations (Sabath and Robles-Osorio, 2012). The presence of hyalinosis casts and accumulation of proteins in the renal tubules, confirmed by the special PAS staining, was evidenced in free-living terrestrial mammals in cases of exposure to heavy metals, such as Mercury, which even affect the structure of the glomeruli (Sonne et al., 2007). Also, Cadmium and Lead are elements that have a remarkable nephrotoxic capacity, affecting the distribution and function of proteins, also causing many changes such as those observed in our study, such as hyperemia, tubular cell swelling, inflammatory infiltrate, abnormalities in the glomeruli and tubular lesions. (Yuan et al., 2014). Our results also showed glomerular dilatation, tubular degeneration and atrophy, changes described in a population of arctic foxes exposed to pesticides (Sonne et al., 2008). As irrigated rice is planted annually in the area sampled in our study, and *H. chacarius* were captured at the edge of the planting area, most likely the observed renal lesions could be a consequence of the pesticides used in the crop.

The hydropic degeneration evidenced by intracytoplasmic vacuolization seen in the liver of 27.3% of the evaluated animals is normally associated with causes such as failure in cellular energy production or alteration in enzyme function, but it can also have secondary causes such as exposure to toxins, bacteria, virus or free radical action (Myers et al., 2012). In a report of controlled intoxication in the laboratory with rainbow trout, animals exposed to biotoxins showed only hepatocytes vacuolization, however, when small mammals were studied, vacuolization was associated with the presence of hemorrhage (KOTAK, et al., 1995 and 1996). In Iran, free-living rodents exposed to heavy metals such as: Cadmium, Copper, Lead and Iron presented intracytoplasmic vacuolization as the most common histopathological lesion (Shahsavari et al., 2019). The presence of hydropic degeneration in the liver can also be associated with the presence of pollutants in the environment (Stehr et al., 1998), since toxic substances can cause oxidative stress in the body and cause injury and cell death (Ekinci-Akdemir et al., 2020). The presence of nematodes in the liver parenchyma of rodents can also cause vacuolization of the cells, and compromise a large part of the tissue with the presence of the parasite (Resendes, et al. 2009), as observed in the two individuals parasitized by *Capillaria*.

The polymorphonuclear inflammatory infiltrate found in the skin of the abdomen was considered non-specific, since they are free-living animals and the reaction was mild to moderate in 15 animals. Inflammatory responses in the skin can be caused by various stimuli, which can end up altering the skin's microbiota, such as exposure to bacteria found in the environment (Gimblet, et al. 2017). Fungi can also be the cause of these infections in the skin of free-living small mammals, especially those species that inhabit aquatic environments (Hubalek, 2000). Other causes frequently associated with skin diseases in small rodents and of veterinary importance are: ectoparasites, such as mites and ticks, insects, endocrine disorders, and nutritional deficiencies (Scarff, 1991).

In this study, the effectiveness of histopathological examination as a biomarker was verified, capable of monitoring the effects of the environment on the health of free-living small mammals through the presence of tissue lesions. The histopathological changes serve as an alert system for more serious health problems that may affect the animal according to the time or amount of exposure, so it is a remarkable monitoring tool especially when working with small wild mammals because they have a short life cycle and being in direct contact with the environment (Gerber et al., 2017). In the case of small rodents of the genus *Holochilus*, the species of this semi-aquatic group, live an average of one year and are greatly exposed to heavy metals and environmental pollutants that may be in suspension or in solution in water. Due to daily exposure to various pollutants in the natural environment or not, in the last decade there has been a growing demand for the application of bioindicators that establish the effect of toxics on the body and their potential risk (Chiarelli and Roccheri, 2014). In a histopathological evaluation carried out with the viscera of fish exposed to waste from a mining area in western Greenland, in addition to lesions associated with heavy metals, lesions associated with lack of food, endoparasites, oxidative stress and other microorganisms such as bacterias and virus were identified (Sonne et al., 2014). The use of small mammals as bioindicators is ideal, since it is possible to monitor the effects of environmental changes and also to estimate the consequences on the human organism, once observed the proximity of these animals in areas with anthropic action and their significant importance in the food chain of birds and carnivorous mammals (Sánchez-Chardi et al., 2008).

The histopathological lesions observed in this study can be associated in numerous ways with the presence of anthropic action, such as the presence of pigments in the lung, lesions suggestive of exposure to heavy metals and the occurrence of zoonotic parasites. The presence of the genus *Capilaria* found in the liver of two animals is an example of this, since hepatic capillariasis is a common disease in humans and domestic dogs (Camargo et al., 2010; Quadros et al., 2016). This interaction of wild fauna with domestic animals (production and companionship) and humans constitutes a risk factor for the emergence of emerging infectious diseases, since the sanitary condition of the environment is related to animal health and this, in turn, is directly linked with human health (Zanardi et al., 2020). The link between all these factors is presented by the unique health, a concept in which the wild animal is appointed as a sentinel, due to its ability to serve as a reservoir, considering its proximity to humans and other domestic animals (Baptista et al., 2021). In this sense, ecopathology, in the single health approach, can provide important observations of the different interactions that occur in the tissue environment, not only by infectious-parasitic agents but also by environmental contaminants (Faye and Lancelot, 2006; Lau, 2010).

5. Conclusion

Our results demonstrate that the use of the histopathological matrix associated with the analysis of body condition proved to be an effective tool in health verification, since animals with greater intensity of lung lesions presented worse body condition. Furthermore, our results showed that, although we observed severe lesions in the lung and some hepatic and renal impairment, in general, the evaluated animals presented low scores in the histopathological matrix, suggesting that the studied population is in a good state of health.

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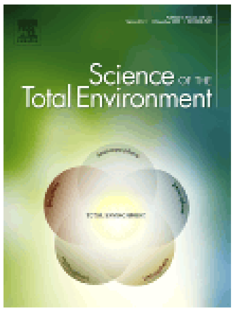

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