

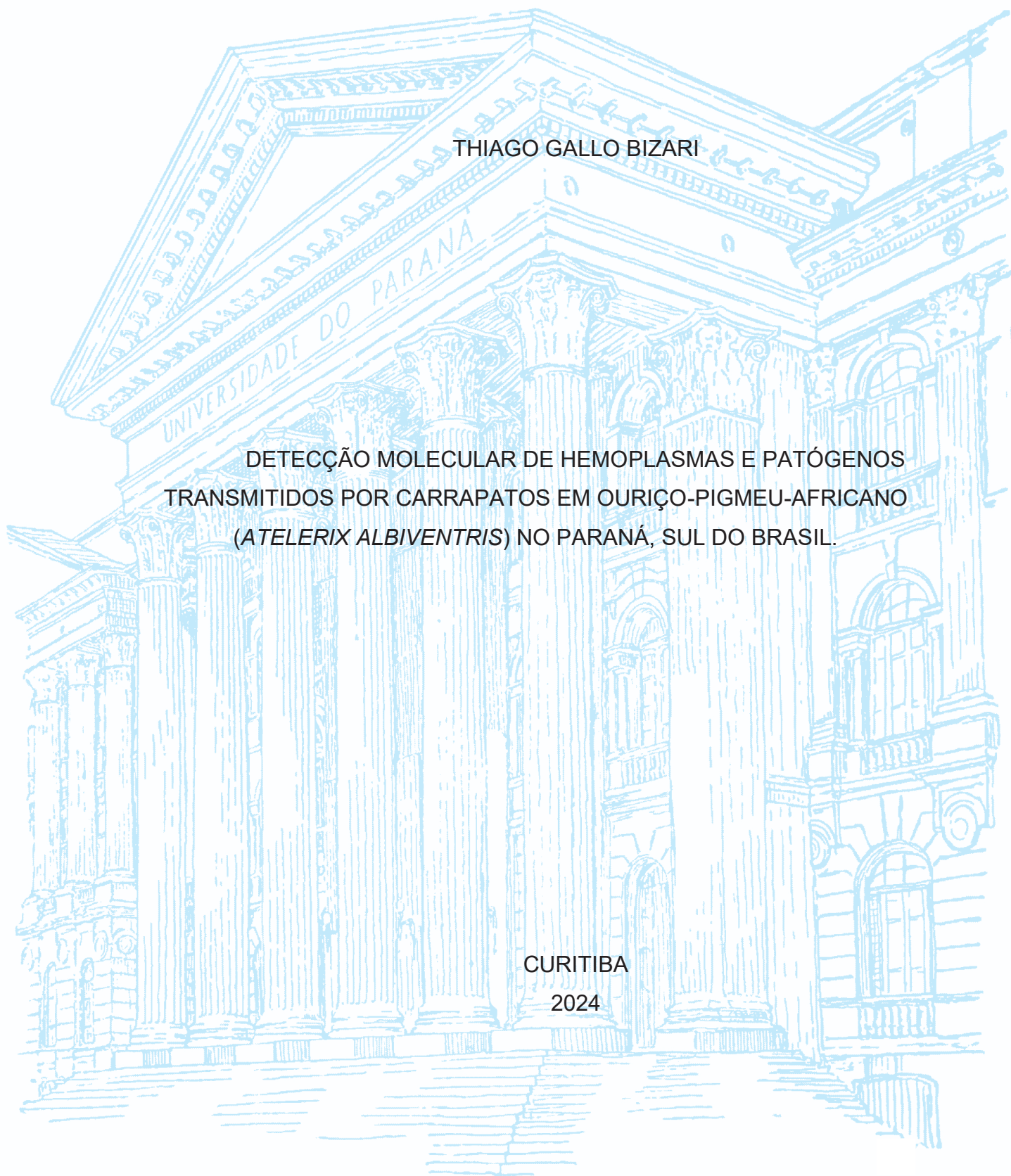
UNIVERSIDADE FEDERAL DO PARANÁ

THIAGO GALLO BIZARI

DETECÇÃO MOLECULAR DE HEMOPLASMAS E PATÓGENOS
TRANSMITIDOS POR CARRAPATOS EM OURIÇO-PIGMEU-AFRICANO
(*ATELERIX ALBIVENTRIS*) NO PARANÁ, SUL DO BRASIL.

CURITIBA

2024



THIAGO GALLO BIZARI

DETECÇÃO MOLECULAR DE HEMOPLASMAS E PATÓGENOS
TRANSMITIDOS POR CARRAPATOS EM OURIÇO-PIGMEU-AFRICANO
(*ATELERIX ALBIVENTRIS*) NO PARANÁ, SUL DO BRASIL.

Dissertação apresentada ao Programa de Pós-graduação em Ciências Veterinárias, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito à obtenção do título de Mestre em Ciências Veterinárias.

Orientador: Prof. Dr. Rafael F. C. Vieira

CURITIBA

2024

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS AGRÁRIAS

Bizari, Thiago Gallo

Detecção molecular de hemoplasmas e patógenos transmitidos por carrapatos em Ouriço-Pigmeu-Africano (*Ateletrix Albiventris*) do Paraná, Sul do Brasil / Thiago Gallo Bizari. – Curitiba, 2024.

1 recurso online: PDF.

Dissertação (Mestrado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciências Veterinárias.

Orientador: Prof. Dr. Rafael F. C. Vieira

1. Medicina veterinária de pequenos animais. 2. Carrapatos.
3. Vetor. I. Vieira, Rafael F. C. II. Universidade Federal do Paraná.
Programa de Pós-Graduação em Ciências Veterinárias. III. Título.

Bibliotecária: Ana Camila Quaresma Moura CRB-9/2212



MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS
VETERINÁRIAS - 40001016023P3

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **THIAGO GALLO BIZARI** intitulada: **DETECÇÃO MOLECULAR DE HEMOPLASMAS E PATÓGENOS TRANSMITIDOS POR CARRAPATOS EM OURIÇO-PIGMEU-AFRICANO (ATELETRIX ALBIVENTRIS) DO PARANÁ, SUL DO BRASIL**, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 03 de Maio de 2024.

Assinatura Eletrônica

25/06/2024 10:42:33.0

FABIANO MONTIANI FERREIRA

Presidente da Banca Examinadora

Assinatura Eletrônica

27/06/2024 10:48:38.0

PHILIPPE VIEIRA ALVES

Avaliador Externo (UNIVERSIDADE ESTADUAL PAULISTA - UNESP)

Assinatura Eletrônica

25/06/2024 14:26:14.0

LUIZ DANIEL DE BARROS

Avaliador Externo (UNIVERSIDADE ESTADUAL DE LONDRINA)

RUA DOS FUNCIONÁRIOS, 1540 - CURITIBA - Paraná - Brasil

CEP 80035050 - Tel: (41) 3350-5621 - E-mail: cpgcv@ufpr.br

Documento assinado eletronicamente de acordo com o disposto na legislação federal Decreto 8539 de 08 de outubro de 2015.

Gerado e autenticado pelo SIGA-UFPR, com a seguinte identificação única: 375374

Para autenticar este documento/assinatura, acesse <https://siga.ufpr.br/siga/visitante/autenticacaoassinaturas.jsp> e insira o código 375374

DEDICATÓRIA

Dedico este trabalho a minha família, que
solidificou a ponte para que eu pudesse sempre seguir meus objetivos sem
maiores preocupações.

AGRADECIMENTOS

Agradecimentos eternos aos meus pais, que sempre deram tudo de si e me incentivaram a seguir meus objetivos, sem nunca recusar ajuda e apoio, seja emocional ou financeiro, desde meus primeiros passos até hoje.

Aos meus amigos, por aliviar os dias difíceis e melhorar os dias alegres, proporcionando sorrisos e ânimo para continuar todos os dias sendo uma pessoa e um profissional melhor.

À todos do Laboratório de Doenças Transmitidas por Vetores, que me ensinaram do básico ao inovador, me auxiliaram durante o período do mestrado, juntos permitiram que todo o trabalho fosse realizado.

Ao meu orientador, Prof. Rafael Vieira, por ter me aceitado entre seus alunos, além de permitido o aprendizado teórico e prático imenso, demonstrado a importância de uma orientação humana e saudável, sendo muito mais que um simples orientador.

Aos órgãos estaduais e federais (CAPES, CNPQ e IAT), por direta ou indiretamente possibilitarem que não apenas este, mas diversos projetos sejam desenvolvidos, permitindo que a pesquisa brasileira continue trazendo resultados.

EPÍGRAFE

“Não existe saber mais, ou saber menos. Há saberes diferentes.”

-Paulo Freire

RESUMO

O mercado de *pets* não convencionais cresce cada vez mais, e com isso observa-se novos animais selvagens que começam a ser enquadrados nesse setor. Entre eles, há uma popularidade crescente pelo ouriço-pigmeu-africano *Atelerix albiventris* (MAMMALIA: ERINACEIDAE). Estes animais são originários do continente africano, sendo uma espécie exótica no Brasil. Por seu potencial invasor, assim como o atual conhecimento de seu papel como transmissor de doenças, é importante compreender como estes animais se comportam na cadeia epidemiológica de diversas doenças. O objetivo deste trabalho foi caracterizar um perfil hematológico, bioquímico e de microbiota cutânea e fecal, assim como pesquisar agentes infecciosos por meio de técnicas moleculares. Amostras de sangue, fezes e swab de pele foram coletadas de vinte e cinco (25) ouriços apreendidos no estado do Paraná. Destas, vinte e duas (22) amostras de sangue foram selecionadas e submetidas a análises moleculares por ensaios de PCR convencional para *Theileria/Babesia* spp. (18S rRNA) e *Ehrlichia/Anaplasma* spp e PCR em tempo real (qPCR) para *Mycoplasma* spp. (16S rRNA). (16S rRNA). Uma parte do sangue coletado para avaliação hematológica apresentou coágulo e fibrina, dificultando a avaliação de alguns parâmetros. Os dados hematológicos e bioquímicos estão sumarizados nas Tabelas 1 e 2, consecutivamente. Os exames microbiológicos da pele mostraram crescimento de *Staphylococcus* spp. (25/25) e *Streptococcus* spp. (13/25). Nas fezes, o isolamento de *Candida tropicalis* (15/25), *Mucor* spp. (12/25) e *Proteus vulgaris* (10/25) foi proeminente. A avaliação molecular dos 22 ouriços resultou negativa para os genes pesquisados, indicando que estes animais não estavam infectados pelos patógenos estudados. Uma vez que são animais mantidos sob tratamento humano, é possível que não tenham tido contato prévio com o vetor destas doenças, ou até mesmo que tenham um protocolo sanitário terapêutica/preventivo realizado frequentemente. Buscar compreender a cadeia de transmissão e determinar protocolos para anteceder doenças nesses animais se faz necessário, uma vez que programas de vigilância são necessários para mitigar ações de saúde pública eficientes e evitar grandes danos ao ambiente e a saúde dos humanos e animais.

Palavras-chave: hedgehogs; carrapatos; doenças transmitidas por vetores.

ABSTRACT

The market for unconventional pets is growing more and more, and as a result, new wild animals are beginning to be included in this sector. Among them, there is growing popularity for the African pygmy hedgehog *Atelerix albiventris* (MAMMALIA: ERINACEIDAE). These animals originate from the African continent, being an exotic species in Brazil. Due to their invasive potential, as well as the current knowledge of their role as disease transmitters, it is important to understand how these animals behave in the epidemiological chain of various diseases. The objective of this work was to characterize a hematological, biochemical and cutaneous and fecal microbiota profile, as well as research infectious agents using molecular techniques. Blood, fecal, and skin swab samples were collected from twenty-five (25) hedgehogs seized in the state of Paraná. Twenty-two (22) blood samples were selected and subjected to molecular analysis by conventional PCR assays for *Theileria/Babesia* spp. (18S rRNA) and *Ehrlichia/Anaplasma* spp and real-time PCR (qPCR) for *Mycoplasma* spp. (16S rRNA). (16S rRNA). Some of the blood collected for hematological evaluation showed clots and fibrin, making it difficult to evaluate some parameters. Hematological and biochemical data are summarized in Tables 1 and 2, consecutively. Microbiological examinations of the skin showed growth of *Staphylococcus* spp. (25/25) and *Streptococcus* spp. (25/13). In the feces, the isolation of *Candida tropicalis* (15/25), *Mucor* spp. (25/12) and *Proteus vulgaris* (25/10) were prominent. The molecular evaluation of the 22 hedgehogs was negative for the genes studied, indicating that these animals were not infected by the pathogens studied. Since they are animals kept under human treatment, it is possible that they have not had previous contact with the vector of these diseases, or even that they have a therapeutic/preventive health protocol frequently carried out. Seeking to understand the transmission chain and determine protocols to precede diseases in these animals is necessary, since surveillance programs are necessary to mitigate efficient public health actions and avoid major damage to the environment and the health of humans and animals

.Keywords: hedgehogs; ticks; tick-borne-disease.

LISTA DE ABREVIATURAS

CHGM - Average globular hemoglobin

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid

Gapdh - Glyceraldehyde 3-fosfate dehydrogenase gene

Hb - Hemoglobin's concentration

Hm - Hematimetry

IAT – Instituto Água e Terra

IBGE – Instituto Brasileiro de Geografia e Estatística

IQR – Interquartile Range

MCHC – Mean Corpuscular Haemoglobin Concentration

MCV – Mean Corpuscular Haemoglobin

NaCl – Cloreto de sódio

PCR – Polymerase Chain Reaction

PCV – Packed cell volume

PIB – Produto Interno Bruto

PPT – Proteína plasmática total

RNA – Ribonucleic acid

s – Desvio padrão

VGM - Average globular volume

$\bar{x}$ - Média

SUMÁRIO

1. INTRODUÇÃO.....	12
2. REVISÃO BIBLIOGRÁFICA	12
2.1 Saúde pública.....	12
2.2 Animais silvestres.....	13
2.3 Ouriços-pigmeus-africanos (<i>Atelerix albiventris</i>).....	14
2.4 Patógenos	14
3. REFERÊNCIAS	16
4. Objetivos	18
4.1 Objetivo Geral	18
4.2 Objetivos Específicos	18
5. Artigo: Molecular screening of tick-borne pathogens and hemotropic mycoplasmas in captive african-pygmy-hedgehogs (<i>Atelerix albiventris</i>) from the state of Paraná, south Brazil	19
Abstract.....	19
Introduction	19
Material and Methods	20
Sampling	20
Blood samples.....	20
Complete blood count and biochemical analysis	21
DNA extraction and PCR assays.....	211
Skin swabbing	212
Feces culture.....	22
Statistical analysis	22
Results	223
Blood count and biochemical analysis.....	233
PCR results.....	23
Skin swabbing.....	23

Feces culture	233
Discussion	234
Conclusion	246
Acknowledgements	266
Conflict of interest.....	266
References.....	26
6. CONSIDERAÇÕES FINAIS	33

1. INTRODUÇÃO

A interação humano-animal vem aumentando cada vez mais, fazendo com que haja um contato próximo entre eles e permitindo que patógenos circulem interespecies. A identificação de zoonoses e o entendimento da epidemiologia destes agentes é de suma importância para criar políticas de controle e prevenção destas doenças (Natterson-Horowitz et al., 2022).

Dentre as zoonoses, as doenças transmitidas por vetores possuem grande relevância em decorrência da ampla disposição geográfica de seus vetores e fácil disseminação, uma vez que havendo os meios adequados, o ciclo se completa. Agentes como bactérias, vírus e protozoários se enquadram nestas categorias e possuem grande relevância na saúde pública (Madison-Antenucci et al., 2020). Muitas vezes estas doenças são negligenciadas e subdiagnosticadas, enquanto isso seus números aumentam e ações efetivas de controle não são colocados em prática.

Além disso, o crescente mercado de pet exóticos coloca em foco a discussão sobre as doenças que podem ser compartilhadas entre os animais e os humanos. Os ouriços-pigmeus-africanos (*Atelerix albiventris*), conhecidos como hedgehogs, tem popularidade crescente no Brasil, apesar de seu comércio ainda não ser autorizado pelos órgãos legais.

A participação destes animais na epidemiologia de doenças é ainda pouco conhecida, havendo poucos dados no mundo com a detecção de agentes infecciosos e as alterações clínicas causadas nestes animais. (Riley & Chomel, 2005).

2. REVISÃO BIBLIOGRÁFICA

2.1 Saúde pública

Zoonoses são doenças que afetam os seres humanos e são transmitidas por animais, relacionadas muitas vezes com classes sociais baixas e áreas menos urbanizadas. Muitas destas doenças acabam não sendo diagnosticadas e favorecem a subnotificação (Natterson-Horowitz et al., 2022).

A expansão das áreas urbanas para onde antes havia mata nativa possibilita maior proximidade entre animais selvagens e os humanos, o que além de aumentar a transmissão de doenças entre ambos, favorece interações que muitas vezes podem causar o óbito dos animais (Richini-Pereira et al., 2010).

Os agentes transmitidos por vetores possuem grande relevância quando falamos de zoonoses. Encontrando o vetor no ambiente, as chances de disseminação da doença são muito grandes (Dantas-Torres et al., 2012). Doenças como Leishmaniose, Febre amarela e Doença de Lyme ganharam destaques nos últimos anos e afetam milhares de indivíduos anualmente (Gorem et al, 2023).

Entender como esses agentes se comportam, quais os possíveis hospedeiros e o papel na transmissão entre humanos são essenciais para que políticas públicas sejam criadas e colocadas em prática, visando diminuir os casos e controlar possíveis endemias. Para tanto, é necessário que a devida atenção seja dada e diagnósticos corretos sejam realizados (Richini-Pereira et al., 2010).

2.2 Animais silvestres

A presença de agentes zoonóticos em animais silvestres torna-se importante quando vemos a população humana interagindo cada dia mais com áreas devastadas e entrando em contato com a fauna (Bicca-Marques e De Freitas, 2010). Em alguns casos, doenças que não apresentam manifestação nestes animais acabam causando sinais clínicos nos animais domésticos e em humanos.

Entender como estas espécies coexistem nos ajuda a prever aumentos de casos e muitas vezes servem como sentinelas de doenças emergentes. Macacos, por exemplo, são grandes indicadores do aumento de casos de febre amarela e ajudam a nortear políticas para evitar que os casos em humanos não cresçam exponencialmente (Bicca-Marques e De Freitas, 2010).

Quando pensamos nos animais silvestres exóticos que se tornam pets, temos um problema ainda maior levando em consideração que esses animais muitas vezes são espécies com potencial para se tornar invasoras e quebrar o equilíbrio do nosso ecossistema (Lockwood et al, 2019). Ainda, a falta de acompanhamento médico-veterinário e o fato de muitas vezes serem espécies ilegais dificulta o controle sanitários sobre esses animais, dificultando medidas de controle e prevenção da disseminação de diversas doenças.

Esses pets exóticos acabam se tornando grande fonte de dispersão de zoonoses conhecidas e em alguns casos, permitem que novos agentes entrem em contato e se tornem problemas de saúde pública (Chomel et al, 2007), aumentando

não apenas os riscos, mas também favorecendo o desenvolvimento de doenças novas e mais adaptadas.

2.3 Ouriços-pigmeus-africanos (*Atelerix albiventris*)

Os ouriços pigmeus africanos (*A. albiventris*), também conhecidos como hedgehogs, são animais encontrados normalmente no continente africano, sendo disseminado pelo mundo como um pet não convencional. É o menor entre os animais do gênero *Atelerix*, tendo comportamento onívoro e noturno predominantemente. (Santana et al, 2010)

A relação destes animais com diversos patógenos que acometem outros animais e são transmitidas por vetores está cada vez mais em pauta. A identificação destes micro-organismos, associando eles a doenças clínicas e buscar correlacionar com a epidemiologia destas doenças se faz necessária para compreender o papel dos hedgehogs na transmissão de zoonoses (Riley & Chomel, 2005).

2.4 Patógenos

Os hemoparasitos da ordem Piroplasmida (*Babesia*, *Cytauxzoon* e *Theileria*) possuem relevância epidemiológica. São organismos intracelulares obrigatórios que necessitam de um hospedeiro vertebrado e invertebrado para concluir seu ciclo de vida. Estes agentes muitas vezes causam sinais clínicos em animais domésticos, mas costumam ser assintomáticos em animais selvagens. (Schnittger et al., 2022).

Os protozoários do gênero *Babesia* comumente são encontrados nos sangues de mamíferos. São transmitidos por várias espécies de carrapatos da família Ixodidae, nos quais também realizam parte do ciclo biológico (Scott e Scott, 2018). Estes agentes infectam os eritrócitos dos hospedeiros definitivos, levando em alguns casos há anemias secundárias a captura destas hemácias.

Os organismos do gênero *Cytauxzoon* acometem mais comumente felinos selvagens e costumam causar doenças mais graves nos felinos domésticos. Novas espécies são frequentemente identificadas em todo o mundo (Gallusová et al., 2016). Com parasitismo em macrófagos, cada vez mais são reportados casos de infecção vinda da fauna silvestre (Squarre et al., 2020).

O gênero *Theileria* se distingue por infectar inicialmente leucócitos e posteriormente eritrócitos. Assim como os anteriores, são transmitidos por carrapatos

e acometem diferentes espécies animais. Com a evolução do diagnóstico molecular, aumentou-se o número de casos, uma vez que na microscopia muitas vezes os organismos eram confundidos entre os diferentes gêneros (Mans et al., 2015).

3. REFERÊNCIAS

- BICCA-MARQUES, J. C.; DE FREITAS, D. S. The role of monkeys, mosquitoes, and humans in the occurrence of a yellow fever outbreak in a fragmented landscape in south Brazil: protecting howler monkeys is a matter of public health. v. 3, n. 1, p. 78-89, 2010.
- CHOMEL, Bruno B.; BELOTTO, Albino; MESLIN, François-Xavier. Wildlife, exotic pets, and emerging zoonoses. *Emerging infectious diseases*, v. 13, n. 1, p. 6, 2007. DANTAS-TORRES, F.; CHOMEL, B. B.; OTRANTO, D. Ticks and tick-borne diseases: a One Health perspective. v. 28, n. 10, p. 437-446, 2012.
- DE SOUSA, K. et al. Anaplasmatidae agents among wild mammals and ectoparasites in Brazil. v. 145, n. 16, p. 3424-3437, 2017.
- GALLUSOVÁ, M. et al. Cytauxzoon infections in wild felids from Carpathian-Danubian-Pontic space: further evidence for a different Cytauxzoon species in European felids. v. 102, n. 3, p. 377-380, 2016.
- GOREN, Asena et al. Demographic patterns in Lyme borreliosis seasonality over 25 years. *Zoonoses and Public Health*, v. 70, n. 7, p. 647-655, 2023.
- IBGE, Diretoria de Pesquisas, Coordenação de Trabalho e Rendimento, Pesquisa Nacional por Amostra de Domicílios Contínua - PNAD Contínua - 2019
- LOCKWOOD, Julie L. et al. When pets become pests: the role of the exotic pet trade in producing invasive vertebrate animals. *Frontiers in Ecology and the Environment*, v. 17, n. 6, p. 323-330, 2019. MADISON-ANTENUCCI, S. et al. Emerging tick-borne diseases. v. 33, n. 2, p. e00083-18, 2020.
- MANS, B. J. et al. A review of Theileria diagnostics and epidemiology. v. 4, n. 1, p. 104-118, 2015.
- NATTERSON-HOROWITZ, B. et al. Beyond Zoonoses in One Health: Non-communicable Diseases Across the Animal Kingdom. p. 2439, 2022.
- RICHINI-PEREIRA, V. et al. Road-killed wild animals: a preservation problem useful for eco-epidemiological studies of pathogens. v. 16, n. 4, p. 607-613, 2010.
- RILEY, P. Y., & CHOMEL, B. B. J. E. I. D. (2005). Hedgehog zoonoses. 11(1), 1.
- SANTANA, Erica M.; JANTZ, Holly E.; BEST, Troy L. *Atelerix albiventris* (Erinaceomorpha: Erinaceidae). *Mammalian Species*, v. 42, n. 857, p. 99-110, 2010.
- SCHNITTGER, L. et al. The Piroplasmida Babesia, Cytauxzoon, and Theileria in farm and companion animals: Species compilation, molecular phylogeny, and evolutionary insights. p. 1-39, 2022.

SCOTT, J. D.; SCOTT, C. M. Human babesiosis caused by *Babesia duncani* has widespread distribution across Canada. Healthcare, 2018, Multidisciplinary Digital Publishing Institute. p.49.

SOARES, J. F. et al. Natural infection of the wild canid, *Cerdocyon thous*, with the piroplasmid *Rangelia vitalii* in Brazil. v. 202, n. 3-4, p. 156-163, 2014.

SQUARRE, D. et al. Investigation of the piroplasm diversity circulating in wildlife and cattle of the greater Kafue ecosystem, Zambia. v. 13, n. 1, p. 1-11, 2020.

SZEKERES, S. et al. Road-killed mammals provide insight into tick-borne bacterial pathogen communities within urban habitats. v. 66, n. 1, p. 277-286, 2019.

4. Objetivos

4.1 Objetivo Geral

O presente estudo teve por propósito pesquisar a infecção de *Atelerix albiventris*, uma espécie exótica no Brasil, por agentes zoonóticos comumente encontrada em nosso território e determinar um perfil sanitário.

4.2 Objetivos Específicos

- a) Pesquisar patógenos transmitidos por carrapatos e hemoplasmas em *A. albiventris*;
- b) Caracterizar o perfil hematológico e bioquímico dos *A. albiventris*
- c) Caracterizar a microbiota cutânea e fecal dos *A. albiventris*

5. Artigo: Health profile of captive Four-toed hedgehogs (*Atelerix albiventris*) from Paraná state, southern Brazil

Abstract

Four-toed hedgehogs are omnivorous mammals native to sub-Saharan Africa and popular as pets worldwide. In Brazil, while their trade is illegal, confiscations remain frequent. This study aimed to assess the health status of 25 hedgehogs from illegal breeding sites, including hematological and biochemical analysis, as well as fecal and skin pathogen screenings, and to investigate the occurrence of haemotropic *Mycoplasma* spp. and tick-borne pathogens in these animals. Real-time PCR (qPCR) was used to detect the 16S rRNA gene of hemoplasmas, while conventional PCR targeting 18S rRNA and 16S rRNA genes were used to identify *Theileria/Babesia* spp. and *Ehrlichia/Anaplasma* spp, respectively. No ectoparasites were found in these animals, and the hematological and biochemical evaluation indicated a good general state of health. Microbiological examinations of the skin showed growth of *Staphylococcus* spp. (25/25) and *Streptococcus* spp. (13/25). In the feces, the isolation of *Candida tropicalis* (15/25), *Mucor* spp. (12/25) and *Proteus vulgaris* (10/25) were prominent. All samples tested negative for *Mycoplasma* spp., *Theileria/Babesia* spp., and *Ehrlichia/Anaplasma* spp. The data obtained suggest that the animals evaluated were not infected by harmful pathogens. Understanding their role in zoonotic transmission is necessary, especially when about exotic species.

Keywords: *Ehlichia* spp.; *Anaplasma* spp.; *Mycoplasma* spp.; Hedgehog.

Introduction

Four-toed hedgehogs (*Atelerix albiventris*, Mammalia: Erinaceidae) are nocturnal, omnivorous mammals widely distributed across sub-Saharan Africa (Harrison & Bates 1991; Kock & Ebenau 1996; Hutterer 2005) and are considered an exotic species in Brazil. European and African hedgehogs are increasingly sought as exotic companion animals (Ruszkowski et al., 2021), although keeping them without special permits is illegal in several countries. Despite the prohibition on the commercialization and ownership of hedgehogs in Brazil, confiscation reports are

frequent, underscoring the need for policies to prevent their uncontrolled breeding and spread.

Hedgehogs are commonly infested by a diversity of ectoparasites such as hard ticks (Acari: Ixodidae) and fleas (Souza et al., 2006) which are regarded as vectors of zoonotic pathogens, such as *Rickettsia* sp., *Borrelia* sp., *Ehrlichia* sp., *Anaplasma* sp. and *Bartonella* sp. species (Bezerra-Santos et al., 2021), *Leishmania* sp. (Pourmohammadi and Mohammadi-Azni, 2019) and tick-borne encephalitis viruses. Considering the role of other animals of the Erinaceidae family in disseminating these agents, there is a potential risk for infection in these species as well. Investigating hedgehog pathogens is essential to identify clinicopathological changes (Balti et al., 2021), and detecting the gastrointestinal parasites helps clarify these animals' sanitary and epidemiological profiles. Furthermore, the close relationship between humans and animals raises concerns about the potential of zoonotic diseases. Also, the increasing demand for unconventional pets for companionship presents a challenge to public health, especially regarding diseases that are often overlooked (Riley & Chomel, 2005). Therefore, this study aimed to investigate the presence of haemotropic Mycoplasma and tick-borne pathogens (TBP), such as Ehrlichia/Anaplasma and Babesia/Theileria, while also evaluating the hematological and biochemical profiles of four-toed hedgehogs apprehended by the Água e Terra Institute (IAT), in the state of Paraná, southern Brazil.

Material and Methods

Sampling

A total of 25 four-toed hedgehogs from a governmental apprehension by request of the Água e Terra Institute (IAT) in the state of Paraná, southern Brazil, were selected for the study while kept under the responsibility of the State agency.

Blood samples

After chemical restrained, with an association of ketamine (5 mg/kg) and xylazine (1 mg/kg), the animals were laid on a flat surface, and blood samples (up to 3 mL) were collected by venipuncture of the proximal jugular or cranial vein and placed into sterile tubes containing EDTA (BD Vacutainer®, Franklin Lakes, NJ, EUA) and

stored at -20°C prior the molecular analysis. Three milliliters were stored in tubes containing a serum separator gel (BD Vacutainer®) and kept at room temperature (25°C) until a visible clot appeared. The samples were centrifuged at $1500 \times g$ for 5 min, serum separated and stored at -20°C for biochemical analysis.

Complete blood count and biochemical analysis

The differential leukocyte count was performed by counting 100 leukocytes on a blood smear. Packed cell volume (PCV) was determined by the microhematocrit method, using capillary tubes and centrifugation at 11,000 RPM for 5 minutes (Farrand, 1976). Hemoglobin (HGB) concentration was measured using the spectrophotometric cyanmethemoglobin method. Total red blood cell (RBC), white blood cell (WBC), platelet counts, and hemoglobin (Hgb) concentrations were measured using a hematology analyzer (BC-2800 Vet, Myndray®). The hematimetric indices, including mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), were calculated using Wintrobe's methodology (1990).

Serum samples were thawed at room temperature before analysis on an automated biochemical analyzer (BS-200, Myndray®), previously calibrated using a commercial control serum (Control Lab®). The following parameters were evaluated using the respective methods: total protein concentration (biuret method, Katal®), albumin (bromocresol green method, Katal®), total globulins (calculated by the difference between the total proteins and albumin), urea (UV enzymatic method of urease-GLDH, Kovalent®), creatinine (Jaffé method, Kovalent®), aspartate aminotransferase (AST; UV kinetic method of AST, Katal®), alanine aminotransferase (ALT; UV kinetic method), and alkaline phosphatase (UV kinetic method).

DNA extraction and PCR assays

The genomic DNA from 200 μL of whole blood was extracted using a commercially available kit (ReliaPrep™ gDNA blood Miniprep System, Promega, Madison, Wisconsin, USA), following the manufacturer's instructions. Ultrapure water was used as a negative control in parallel to monitor cross-contamination. A conventional PCR assay (cPCR) for the mammal endogenous gene glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) was performed to ensure successful DNA extraction (Rebouças et al., 2013). Thereafter, the hedgehog's DNA samples were screened

using a universal SYBR green real-time PCR (qPCR) assay targeting the 16S rRNA gene of hemoplasmas, as previously described (Willi et al., 2009). A standard curve was calibrated using serial dilutions of gBlock™ (Integrated DNA Technologies, Coralville, IA, USA). All parameters were analyzed according to the standards established by MIQE (Minimum Information for Publication of Quantitative Real-time PCR Experiments) (Bustin et al., 2009). Additionally, DNA samples from hedgehogs were screened using cPCR assays targeting a 551bp fragment of the 18S rRNA gene of *Theileria/Babesia* spp. (Almeida et al., 2012), and a 349bp fragment of the 16S rRNA gene of *Ehrlichia/Anaplasma* spp. (Parola et al., 2000). DNA from *Babesia vogeli* and *Ehrlichia canis* obtained from naturally infected dogs and nuclease-free water were used as positive and negative controls, respectively.

Skin swabbing

The skin samples of 25 animals were individually collected by swabbing the interscapular skin with sterile flexible rods. These swabs were then plated onto a blood agar plate. Colonies that grew were further identified by light microscopy and biochemical tests following the methodology described by Koneman et al (2006).

Feces culture

25 feces samples were collected from the coop, without isolation of each animal, packed in sterile tubes, and sent for microbiological isolation. The samples were inoculated into tubes containing 2mL of BHI broth (Brain-Heart infusion), incubated under shaking at 37°C overnight. Subsequently, aliquots were plated on MacConkey agar plates supplemented with 2 µg/mL of ceftriaxone and colistin. Isolated colonies were seeded again on McConkey agar plates without antibiotics and incubated at 37°C for 18 hours. The collected colonies were evaluated under optical microscopy and for morphological identification. (Koneman et al., 2006)

Statistical analysis

The Chi-square test was used to determine the average values and deviations from the standard hematological patterns. A 95% confidence interval and p-values were calculated. For samples rejected normality (RN), robust data analysis was

performed, and the interquartile range (IQR) was calculated. Data compilation and analysis were performed using Microsoft Excel™ and the MedCalc® software.

Results

Blood count and biochemical analysis

The mean ($\bar{x}$) and standard deviation (s) of the blood counting and hematocrit were $5.9:0.99 \times 10^{12}/L$ and $40.7:4.64\%$, respectively. The statistical analysis of the blood counting and biochemistry results are summarized in Tables 1 and 2, respectively.

After evaluation, some samples were excluded from the white blood series counting and differential due to the presence of clogs. Additionally, some samples had insufficient volume to repeat or confirm some biochemical tests and were excluded from the results.

PCR results

The *gapdh* gene was consistently amplified in all hedgehog samples. All samples tested negative for hemoplasmas, *Ehrlichia/Anaplasma* spp., and *Theileria/Babesia* spp.

Skin swabbing

Nine pathogens were detected in the animals, with some cases exhibiting co-infections. *Candida tropicalis* (15/25, 60%; 95% CI), *Mucor* spp. (12/25, 48%; 95% CI), *Proteus vulgaris* (10/25; 40%; 95% CI) and *Enterobacter sakazakii* (7/25; 28%; 95% CI) were the most frequent pathogens, followed by *Proteus mirabilis* (4/25; 16%; 95%CI), *Citrobacter amalonaticus* (2/25; 8%; 95% CI), *Enterobacter* spp. (2/25; 8%; 95% CI:), *Geotrichum* spp. (1/25, 4%; 95% CI), and *Aspergillus* spp. (1/25, 4%; 95% CI).

Feces culture

From the 30 fecal samples, specimens of *Bacillus* spp. (4/25; 3,3%; 95% CI), *Micrococcus* spp. (2/25; 8%; 95% CI); *Staphylococcus* spp. (25/25; 100%; 95% CI), and *Streptococcus* spp. (13/25; 52%; 95% CI) were identified.

Discussion

In microbiological examinations of the skin, the detection of *Candida* spp. in 60% of patients is relevant when considering the zoonotic nature of this pathogen, more effectively in the treatment of direct handlers, such as zookeepers and veterinarians, and in the case of contact with immunosuppressed individuals. Furthermore, the other pathogens identified may have been found in skin diseases or even systemic diseases as secondary opportunistic agents, since they appear to be present on the skin.

In an analysis of vaginal swabs from some sea urchins in Indonesia, specimens of *Proteus* spp. and *E. coli* were isolated (Ash-Santri et al, 2021). When compared with our data from skin swabs, there is a greater variety of agents present in the skin than in the genital tract.

The fecal analysis yielded a different result from another study in Indonesia (Amanbayeva, 2021). This could be associated with the difference in location and the consequent change in the microbiota. *Salmonella* spp. has been described in *A. albiventris* (Perez et al., 2021), causing concern about the role of these animals in its transmission. In the present study, this bacterium was not observed, which may again be related to the environment in which the evaluated animals were found.

On the other hand, the observation of *Staphylococcus* spp. in all samples, as well as *Streptococcus* spp. in 52% of the samples, shows an incidence of these agents in the digestive tract of these animals, which may also act as pathogens in cases of dysbiosis or even affect contacts of their species or of different species that are immunocompromised.

The hematological values of four-toe hedgehogs analyzed in this study showed greater variations compared to previous studies (Okorie-Kanu et al., 2014). Although all animals were clinically healthy, some cases of leukocytosis were observed, suggesting an active inflammatory process. The difficulty in determining the exact age of the animals and the limited literature on the hematological patterns of these species complicated the accurate interpretation of the data obtained. Additionally, the serum biochemical analyses showed discrepancies when compared to studies on this species or similar ones (Okorie-Kanu et al., 2015; Gabriele Rossi et al., 2014). For example, compared to other domestic animals, elevated AST values in one of the sampled hedgehogs may indicate significant liver damage or muscle

damage, especially related to increased CK activity. Similarly, the mild hypoalbuminemia observed in this study also suggests the presence of an inflammatory process since albumin is a negative acute-phase protein. During inflammation, the synthesis of specific globulins (acute-phase proteins) increases by hepatocytes, while B lymphocytes synthesize immunoglobulins, leading to a relative decrease in albumin levels (Stocham & Scott, 2011).

Hedgehogs have been suggested as potential hosts or reservoirs of zoonotic vector-borne pathogens (Krawczyk et al., 2015) and are frequently infested with ticks and fleas (Iacob & Iftinca, 2018). The ticks commonly found on hedgehogs belong to the genera *Amblyomma*, *Rhipicephalus*, *Dermacentor*, *Hyalomma*, and *Ornithodoros* (Estrada-Peña et al., 2018; Guglielmone et al., 2014), all of which are known vectors of zoonotic pathogens such as *Rickettsia* spp., *Borrelia* spp., *Ehrlichia* spp., *Anaplasma* spp., and *Bartonella* spp. (Regnery et al., 1992; Bouyer et al., 2001; Millán et al., 2019). These pathogens are of significant medical and veterinary importance in neotropical regions (Barros-Battesti et al., 2006).

Wild animals, mainly those presenting synanthropic behavior, are considered important reservoirs of zoonotic pathogens (Hassell, Begon, Ward, & Fèvre, 2017). In Brazil, there are several studies identifying tick-borne diseases in synanthropic animals, some with great zoonotic importance (Collere et al., 2022; Orozco et al., 2022; Vieira et al., 2009). In a previous study, *A. phagocytophilum* and *Borrelia* spp. were detected in ticks collected from European hedgehogs (*Erinaceus europaeus*) (Krawczyk et al., 2015). Despite the detection of various zoonotic pathogens (bacterial, viral, protozoal, and fungal) in hedgehogs in previous studies (Riley & Chomel, 2005; Dumitrache et al., 2013; Kocan et al., 2010; Krawczyk et al., 2015; Pourmohammadi & Mohammadi-Azni, 2019), all animals in this study tested negative for hemoplasmas and tick-borne pathogens.

Hedgehogs, which thrive in urban, rural, and natural environments alongside domestic animals and humans (Skuballa et al., 2007), can contribute to environmental disturbance when introduced into new ecosystems. Since the hedgehogs were seized animals from captive breeding environment, they may not have been exposed to arthropods that could have infected them. In general, animals bred for trade are often subjected to veterinary care and medications, which may help

maintain a controlled health status. This could explain the absence of pathogens and ectoparasites in the animals studied.

5.0 Conclusion

The present study collected hematological and biochemical data that indicated a certain degree of active inflammation, despite the absence of clinical signs and perceptible clinical disease. It is likely that these animals have greater resistance to small physiological changes. Furthermore, the results of fecal and skin cultures show a diverse microbiota, with possible opportunistic pathogens for the individual or contacts. Vector-borne diseases were not identified. It is necessary to expand scientific knowledge about the hematological and biochemical profiles, as well as the diseases that affect *A. albiventris*, to better understand its unique health and the role of zoonotic diseases in this species.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The Brazilian National Council of Scientific and Technological Development (CNPq) provided a fellowship of research productivity (PQ) to Dr. Rafael F.C. Vieira (CNPq - 313161/2020-8). We appreciate the partnership with the Água e Terra Institute (IAT) and the Environmental Military Police Green Force (police report nº 2021/58132).

Conflict of interest

The authors declare no conflicts of interest.

References

Marcili, A, Leite, RC, Nieri-Bastos, FA, Domingues, LN, Martins, JR, et al. *Coxiella symbiont* in the tick *Ornithodoros rostratus* (Acari: Argasidae). *Ticks and tick-borne diseases* 2012; 3(4): 203-206.

Ash-Santri, AD, Prakasita, VC, Adi, YK, Budipitojo, T, & Wahyuni, AETH. Isolation, Identification, and Antimicrobial Susceptibility Test of Bacteria from Vulva

Swab of African Pygmy Hedgehog (*Atelerix albiventris*) and Sunda Porcupine (*Hystrix javanica*). *BIO Web of Conferences*; 2021 33 06009 doi: 10.1051/bioconf/20213306009

Balti, G, Galon, C, Derghal, M, Souguir, H, Guerbouj, S, Rhim, et al. *Atelerix algirus*, the North African Hedgehog: Suitable Wild Host for Infected Ticks and Fleas and Reservoir of Vector-Borne Pathogens in Tunisia. *Pathogens*. 2021 10(8):953. doi: 10.3390/pathogens10080953.

Barros-Battesti, D. M., Arzua, M., & Bechara, G. H. (2006). *Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para identificação de espécies*. São Paulo Butantan; 2006

Bezerra-Santos, MA, Mendoza-Roldan, JA, Thompson, RA, Dantas-Torres, F, & Otranto, D, Illegal Wildlife Trade: A Gateway to Zoonotic Infectious Diseases. *Trends Parasitol*. 2021 37(3):181-184. doi: 10.1016/j.pt.2020.12.005

Bouyer DH, Stenos J, Crocquet-Valdes P, Moron CG, Popov VL, Zavala-Velazquez JE, et al. *Rickettsia felis*: molecular characterization of a new member of the spotted fever group. *Int J Syst Evol Microbiol*. 2001 51(Pt 2):339-347. doi: 10.1099/00207713-51-2-339.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem*. 2009 55(4):611-22. doi: 10.1373/clinchem.2008.112797.

Collere, FCM, Ferrari, LDR, Drozino, RN, Valente, JDM, Massini, PF, Otomura, FH, et al. Hemotropic mycoplasmas in bats from forest fragments, state of Paraná, southern Brazil. *Semina: Ciências Agrárias*, 2009 43(1), 431-440. doi:10.1590/S1984-296120180074

Dumitrache MO, Paștiu AI, Kalmár Z, Mircean V, Sándor AD, Gherman CM, et al. Northern white-breasted hedgehogs *Erinaceus roumanicus* as hosts for ticks

infected with *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in Romania. *Ticks Tick Borne Dis.* 2013 4(3):214-7. doi: 10.1016/j.ttbdis.2012.11.010.

Estrada-Peña, A, Mihalca, AD, & Petney, TN (Eds.). *Ticks of Europe and North Africa: a guide to species identification*. Springer; 2018.

Farrand LL. The microhematocrit: technique and applications. *Nurse Pract.* 1976 1(5):19-20.

Földvári G, Jahfari S, Rigó K, Jablonszky M, Szekeres S, Majoros G, et al. Candidatus *Neoehrlichia mikurensis* and *Anaplasma phagocytophilum* in urban hedgehogs. *Emerg Infect Dis.* 2014 20(3):496-8. doi: 10.3201/eid2003.130935.

Guglielmone, AA, Robbins, RG, Apanaskevich, DA, Petney, TN, Estrada-Peña, A, & Horak, IG. *The hard ticks of the world*. Springer, 2014.

Harrison DL, Bates PJJ. Felidae. In: Sevenoaks, UK. *The mammals of Arabia*. 2nd ed.: Harrison Zoological Museum; 1991. P. 156-172.

Hassell JM, Begon M, Ward MJ, Fèvre EM. Urbanization and Disease Emergence: Dynamics at the Wildlife-Livestock-Human Interface. *Trends Ecol Evol.* 2017 32(1):55-67. doi: 10.1016/j.tree.2016.09.012.

Hutterer, R, Ivanova, T, Meyer-Cords, C, & Rodrigues, L. Bat migrations in Europe: a review of banding data and literature. BfN-Schriftenvertrieb im Landwirtschaftsverlag, 2005.

Iacob O, Iftinca A. The dermatitis by *Caparinia tripilis* and *Microsporum* , in african pygmy hedgehog (*Atelerix albiventris*) in Romania - first report. *Rev Bras Parasitol Vet.* 2018 27(4):584-588. doi: 10.1590/S1984-296120180053.

Krawczyk AI, van Leeuwen AD, Jacobs-Reitsma W, Wijnands LM, Bouw E, Jahfari S, et al. Presence of zoonotic agents in engorged ticks and hedgehog faeces

from *Erinaceus europaeus* in (sub) urban areas. *Parasit Vectors*. 2015 9;8:210. doi: 10.1186/s13071-015-0814-5.

Kocan KM, de la Fuente J, Blouin EF, Coetzee JF, Ewing SA. The natural history of *Anaplasma marginale*. *Vet Parasitol*. 2010 10;167(2-4):95-107. doi: 10.1016/j.vetpar.2009.09.012.

Kock, D, & Ebenau, C. The desert hedgehog, *Paraechinus aethiopicus* (Ehrenberg, 1833), new to the fauna of Syria. *Zeitschrift fur Saugetierkunde*, 1996 61(3), 189-191.

Koneman EW et al. Color atlas and textbook of diagnostic microbiology. Baltimore: Lippincott Williams & Wilkins. 6a ed., 2006.

Millán J, Travaini A, Cevidanes A, Sacristán I, Rodríguez A. Assessing the natural circulation of canine vector-borne pathogens in foxes, ticks and fleas in protected areas of Argentine Patagonia with negligible dog participation. *Int J Parasitol Parasites Wildl*. 2018 28;8:63-70. doi: 10.1016/j.ijppaw.2018.11.007.

Okorie-Kanu, CO, Onoja, RI, Achegbulu, E.E, & Okorie-Kanu, OJJCCP. Normal haematological and serum biochemistry values of African hedgehog (*Atelerix albiventris*). *Comparative Clinical Pathology* 2015 24(1), 127-132.

Orozco AMO, Bento LD, Souto PC, Girardi FM, Nogueira BCF, Yamatogi RS, et al. '*Candidatus Mycoplasma Haemoalbiventris*' and Tick-Borne Pathogens in Black-Eared Opossum (*Didelphis aurita*) from Southeastern Brazil. *Microorganisms*. 2022 30;10(10):1955. doi: 10.3390/microorganisms10101955.

Parola P, Roux V, Camicas JL, Baradji I, Brouqui P, Raoult D. Detection of ehrlichiae in African ticks by polymerase chain reaction. *Trans R Soc Trop Med Hyg*. 2000 94(6):707-8. doi: 10.1016/s0035-9203(00)90243-8.

Perez, S, Barreto, M, & Retamal, P. Detection of antimicrobial resistant *Salmonella enterica* strains in samples of ground hedgehogs (*Atelerix albiventris*) reared as pets in the urban area of Santiago, Chile. *Austral journal of veterinary sciences*, 2021 53(2), 133-137. doi:10.4067/S0719-81322021000200133

Pourmohammadi B, Mohammadi-Azni S. Molecular Detection of *Leishmania major* in *Hemiechinus auritus*: A Potential Reservoir of Zoonotic Cutaneous Leishmaniasis in Damghan, Iran. *J Arthropod Borne Dis*. 2019 30;13(3):334-343.

Rebouças, EDL, Costa, JJD, Passos, MJ, Passos, JRDS, Hurk, RVD, & Silva, JRV. Real time PCR and importance of housekeeping genes for normalization and quantification of mRNA expression in different tissues. *Brazilian Archives of Biology and Technology* 2013 56, 143-154. doi: 10.1590/S1516-89132013000100019.

Riley PY, Chomel BB. Hedgehog zoonoses. *Emerg Infect Dis*. 2005 11(1):1-5. doi: 10.3201/eid1101.040752.

Regnery RL, Anderson BE, Clarridge JE 3rd, Rodriguez-Barradas MC, Jones DC, et al. Characterization of a novel *Rochalimaea* species, *R. henselae* sp. nov., isolated from blood of a febrile, human immunodeficiency virus-positive patient. *J Clin Microbiol*. 1992 30(2):265-74. doi: 10.1128/jcm.30.2.265-274.1992.

Rossi G, Mangiagalli G, Paracchini G, Paltrinieri S. Hematologic and biochemical variables of hedgehogs (*Erinaceus europaeus*) after overwintering in rehabilitation centers. *Vet Clin Pathol*. 2014 43(1):6-14. doi: 10.1111/vcp.12121.

Ruszkowski JJ, Hetman M, Turlewicz-Podbielska H, Pomorska-Mól M. Hedgehogs as a Potential Source of Zoonotic Pathogens-A Review and an Update of Knowledge. *Animals (Basel)*. 2021 11;11(6):1754. doi: 10.3390/ani11061754.

Silaghi C, Skuballa J, Thiel C, Pfister K, Petney T, Pfäffle M, et al. The European hedgehog (*Erinaceus europaeus*)--a suitable reservoir for variants of

Anaplasma phagocytophilum? *Ticks Tick Borne Dis.* 2012 3(1):49-54. doi: 10.1016/j.ttbdis.2011.11.005.

Skuballa J, Petney T, Pfäffle M, Taraschewski H. Molecular detection of *Anaplasma phagocytophilum* in the European hedgehog (*Erinaceus europaeus*) and its ticks. *Vector Borne Zoonotic Dis.* 2010 10(10):1055-7. doi: 10.1089/vbz.2009.0150.

Stockham, Steven L., and Michael A. Scott. *Fundamentals of veterinary clinical pathology*. John Wiley & Sons, 2011.

De Sousa R, Edouard-Fournier P, Santos-Silva M, Amaro F, Bacellar F, Raoult D. Molecular detection of *Rickettsia felis*, *Rickettsia typhi* and two genotypes closely related to *Bartonella elizabethae*. *Am J Trop Med Hyg.* 2006 75(4):727-31.

Vieira RF, Molento MB, dos Santos LC, Moraes W, Cubas ZS, Santos AP, et al. Detection of a novel hemoplasma based on 16S rRNA gene DNA in captive and free-ranging capybaras (*Hydrochaeris hydrochaeris*). *Vet Microbiol.* 2009 139(3-4):410-3. doi: 10.1016/j.vetmic.2009.06.018.

Willi B, Meli ML, Lüthy R, Honegger H, Wengi N, Hoelzle LE, et al. Development and application of a universal Hemoplasma screening assay based on the SYBR green PCR principle. *J Clin Microbiol.* 2009 47(12):4049-54. doi: 10.1128/JCM.01478-09.

Wintrobe MM. The size and hemoglobin content of the erythrocyte. Methods of determination and clinical application. 1932. *J Lab Clin Med.* 1990 115(3):374-87.

Table 1 – Haematological profile of Captive *Atelerix albiventris* from the state of Paraná, south Brazil

Analyte	UNIT	N	Mean	SD	Median	Min	Max
RBC	10 ¹² /L	25	5.9	0.99	5.68	4.3	7.43
PCV	%	24	40.70	4.64	40.5	33	53
Hemoglobin	g/L	24	120.17	18.68	118.5	92	161
WBC	10 ⁶ /L	17	12.3	5.1	12	6	21.8
Neutrophils	%	17	52.29	12.5	53	32	72
Bands	%	17	0.29	0.58	0	0	2
Lymphocytes	%	17	28.9	9.91	30	16	47
Monocyte	%	17	6.35	4.13	5	1	18
Eosinophils	%	17	9.94	5.91	9	0	19
Basophils	%	17	1.70	1.72	1	0	6
Neutrophils	10 ⁶ /L	17	6.58	3.37	5.73	1.92	13.46
Bands	10 ⁶ /L	17	0.043	0.1	0	0	0.38
Lymphocytes	10 ⁶ /L	17	3.47	1.8	3.36	1.08	8.93
Monocyte	10 ⁶ /L	17	0.78	0.6	0.67	0.12	2.25
Eosinophils	10 ⁶ /L	17	1.16	0.84	0.93	0	3.4
Basophils	10 ⁶ /L	17	0.21	0.22	0.18	0	0.88

Table 2 – Serum biochemistry profile of captive *Atelerix albiventris* from the state of Paraná, south Brazil -

Analyte	UNIT	N	Mean	SD	Median	Min	Max
Urea	mmol/L	22	4.20	0.95	4.04	2.44	6.79
Creatinine	mmol/L	22	40.99	8.43	44.21	26.52	53.05
ALP	U/L	22	64.97	27.41	57.9	10.1	133.9
ALT	U/L	21	67.22	23.89	60.6	32.9	120.7
AST	U/L	21	29.76	14.22	28.6	14.3	80.4
Total protein	g/L	23	69	13.54	69	47	113
Albumin	g/L	23	26.34	5.17	27	10	25
Globulin	g/L	23	43.04	17.46	41	20	112
A:G RATIO		23	0.69	0.25	0.64	0.08	1.35

6. CONSIDERAÇÕES FINAIS

A pesquisa por agentes infecciosos com potencial zoonótico em espécies exóticas que estão sendo inseridas em nosso país é fundamental para evitar grandes danos e prever o comportamento epidemiológico destas doenças nesses animais. Apesar deste estudo não apresentar positividade para os patógenos selecionados, a vigilância passiva deve ser mantida, uma vez que *A. albiventris* é sabidamente uma possível fonte de infecção de diversos patógenos.