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Dissertação

Detecção de DNA de *Trypanosoma* spp. e *Leishmania* spp. em órgãos de javali (*Sus scrofa*)

Bibiana Rodrigues de Freitas

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“O começo de todas as ciências, é o espanto de as coisas serem o que são”
Aristóteles

Resumo

FREITAS, Bibiana Rodrigues. **Detecção de DNA de *Trypanosoma* spp. e *Leishmania* spp. em órgãos de Javali (*Sus scrofa*).** 2023. 40f. Dissertaçção (Mestrado em Ciências) – Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2023.

Os protozoários da família Trypanosomatidae possuem grande importância em saúde única pois são consideradas zoonoses reemergentes, e o javali (*Sus scrofa*) vem se mostrando um potencial disseminador desses agentes. Esta dissertação teve como objetivo avaliar a presença de *Trypanosoma* spp. e *Leishmania* spp. em tecidos de órgãos de javali. Para o desenvolvimento do estudo analisaram-se 75 amostras de 25 animais, provenientes de caça autorizada, sendo as alíquotas enviadas do Laboratório de Imunologia e Microbiologia Veterinária do Instituto Federal Farroupilha (LAMIVET/IFFar) ao Laboratório de Doenças Parasitárias da Universidade Federal de Santa Maria (LADOPAR/UFSM) para análise molecular. Após extração de DNA o material foi submetido à técnica de PCR foi identificado material genético de *Leishmania infantum* (4%) e *Leishmania amazonensis* (4%) em baço e fígado, respectivamente, e de *Trypanosoma* spp. (44%) em onze amostras de coração. Os dados presentes neste estudo revelam a importância do controle populacional de javalis e como estes animais podem servir de reservatório para doenças de relevância em saúde pública.

Palavras-chave: *Trypanosoma* spp.; *Leishmania* spp.; PCR; javali; zoonoses

Abstract

FREITAS, Bibiana Rodrigues. **DNA detection from *Trypanosoma* spp. and *Leishmania* spp. in organs of wild boar (*Sus scrofa*).** 2023. 40f. Dissertation (Master's degree in Science) – Postgraduate degree program in Veterinary Medicine, Faculdade de Medicina Veterinária, Universidade Federal de Pelotas, Pelotas, 2023.

The protozoan of Trypanosomatidae Family have great importance in one health because they are considered reemerging zoonosis, and the wild boar (Sus scrofa) has shown to be a potential disseminator of these agents. The goal of this dissertation was to evaluate the presence of *Trypanosoma* spp. and *Leishmania* spp. in wild boar organ tissues. For the development of the study, 75 samples of 25 animals were analyzed, from authorized hunting, and the aliquots were sent from the (Laboratory of Veterinary Microbiology and Immunology of the Farroupilha Federal Institute of Education (LAMIVET/IFFar) to the Laboratory of Parasitic Diseases of the Federal University of Santa Maria (LADOPAR/UFSM) for molecular analysis. After DNA extraction, the material was submitted to the PCR technique and genetic material for *Leishmania infantum* (4%) and *Leishmania amazonensis* (4%) was identified in the spleen and liver, respectively, and for *Trypanosoma* spp. (44%) in eleven heart samples. The data present in this study reveal the importance of population control in wild boar and how these animals can serve as a reservoir for diseases of public health relevance.

Keywords: *Trypanosoma* spp.; *Leishmania* spp.; PCR; boar; zoonoses.

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Lista de Abreviaturas e Siglas

bp	*Base pairs* (pares de bases)
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CL	*Cutaneous Leishmaniasis* (Leishmaniose cutânea)
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
DNA	*Deoxyribonucleic acid* (Ácido desoxirribonucleico)
dNTP	*Deoxynucleotide Triphosphates* (Desoxirribonucleotídeos Fosfatados)
IFAT	*Indirect Fluorescent Antibody Test* (Reação de imunofluorescência indireta)
IFFAR	Instituto Federal Farroupilha
kDNA	Kinetoplast DNA (DNA do cinetoplasto)
L. amazonensis	*Leishmania amazonensis*
L. infantum	*Leishmania infantum*
LABMol-Vet	Laboratório de Biologia Molecular Veterinária
LADOPAR	Laboratório de Doenças Parasitárias
LAMIVET	Laboratório de Microbiologia e Imunologia Veterinária
MgCl2	Cloreto de Magnésio
Neg	Negativa
Ng	*Nanograms* (nanogramas)
rDNA	*Recombinant DNA* (DNA recombinante)
UFPEL	Universidade Federal de Pelotas
UFSM	Universidade Federal de Santa Maria
VL	*Visceral Leishmaniasis* (Leishmaniose visceral)

Lista de Símbolos

°C	Graus celsius
®	Marca registrada
µL	Microlitros
%	Porcentagem

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1 Introdução

Introduzido no Brasil na década de 80, o javali (*Sus scrofa*) apresenta fácil adaptação nos ambientes, além de uma alta taxa reprodutiva e alimentação diversificada. Com hábitos crepusculares, eles andam em varas compostas por fêmeas adultas e filhotes, os machos abandonam o grupo quando atingem maturidade sexual (BRASIL, 2020).

Devido à falta de predadores naturais, houve um aumento exponencial desta espécie exótica, causando prejuízos ambientais e econômicos, como competição com espécies nativas, depredação do bioma local e disseminação de doenças, sendo algumas consideradas zoonóticas (LOBO, 2022).

Figura 1: Danos causados por javalis em lavoura de milho no Mato Grosso do Sul.
Fonte: Canal Rural.

No Brasil, Carraro (2020) relata a presença de anticorpos para *Toxoplasma gondii*, *Leptospira* spp., *Leishmania infantum* e *Trypanosoma cruzi* em javalis, bem como Soares et al. (2016) encontra anticorpos para e *Neospora caninum*. Já Dib et al. (2020) encontraram larvas de nematódeos, ovos de ascarídeos e nematódeos e protozoários como *Eimeria* sp., *Balantioides coli*, além de coproantígenos para *Cryptosporidium* sp.

Da mesma maneira, autores ao redor do mundo sugerem que o javali age como disseminador de doenças de diversas origens: virais como circovirose suína, diarreia epidêmica suína, parvovírus suíno, vírus da síndrome respiratória e reprodutiva dos suínos, doença de Aujezky; bacterianas como pleuropneumonia, brucelose, leptospirose e enteropatia proliferativa suína; e parasitárias como toxoplasmose, metastrongilose e hidatidose, (CLEVELAND et al., 2017; DI NICOLA et al., 2015).

O trabalho objetivou a pesquisa acerca do javali como potencial transmissor de importantes enfermidades, bem como auxiliar na elucidação de questões como o javali sendo reservatório e disseminador de tais doenças.

2 Revisão de Literatura

2.1 *Trypanosoma* spp.

O gênero *Trypanosoma* faz parte da família Trypanosomatidae e tem como principais características estruturais a presença de um flagelo para sua locomoção e um cinetoplasto com seu DNA (TAYLOR, 2017). Tendo seu ciclo considerado heteroxeno, o este protozoário conta como seus hospedeiros definitivos os vertebrados e os hospedeiros intermediários os invertebrados hematófagos, chamados de vetores, além de seu gênero ser dividido em seção Stercoraria e Salivaria, sendo a segunda de maior relevância e patogenicidade na pecuária (SILVA et al., 2002).

Os parasitos da seção Stercoraria são transmitidos para o hospedeiro definitivo (vertebrados) através das fezes dos hospedeiros intermediários (invertebrados) depositadas próximo à picada, já na seção Salivaria acontece a inoculação direta através da picada (MONTEIRO, 2017). Os sinais clínicos em animais podem incluir perda de peso, anemia, perda de visão, hemorragia, diarreia, aborto e alterações neurológicas, variando de acordo com a espécie de Trypanosoma circulante (CARVALHO et al., 2008; FELIPE & KATAOKA, 2019; MONTEIRO, 2017). Em humanos a tripanosomose pode causar febre, conjuntivite unilateral, miocardite, megacólon, esplenomegalia e demais complicações (RODRIGUES et al., 2020; COURA, 2003).

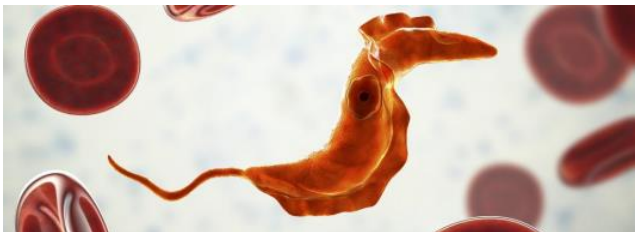

Figura 2: Representação de *Trypanosoma cruzi* na corrente sanguínea.
Fonte: Instituto Nacional de Controle de Qualidade em Saúde/FIOCRUZ.

2.2 *Leishmania spp.*

O gênero Leishmania também apresenta ciclo heteroxeno, dependendo geralmente de flebotomíneos do gênero *Lutzomyia* para a inoculação direta, infectando animais como cães, humanos e animais silvestres, podendo causar dois tipos de doenças em seus hospedeiros definitivos: a leishmaniose cutânea e a leishmaniose visceral (MONTEIRO, 2017; MARTINS, 2019).

Em cães a leishmaniose visceral pode levar à febre intermitente, caquexia, perda de pelos e eczema, já a cutânea pode causar úlceras de recuperação rápida (TAYLOR, 2017). Nos humanos a leishmaniose visceral pode ser causadora de fadiga, febre e esplenomegalia, sendo que a cutânea causa pápula eritematosa, lesões que atingem mucosas e até mesmo lesões nodulares disseminadas pelo corpo todo (FREITAS, 2021).

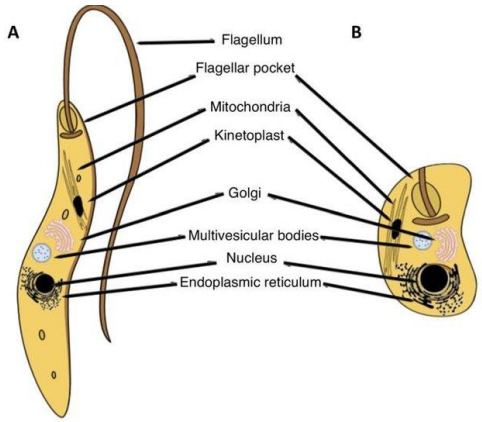

Figura 3: Representação de *Leishmania* em sua forma promastigota (A) e amastigota (B).
Fonte: ResearchGate.

3 Artigo

Molecular detection of DNA from *Trypanosoma* spp. and *Leishmania* spp. in wild boar (*Sus scrofa*) tissues

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Molecular detection of DNA from *Trypanosoma* spp. and *Leishmania* spp. in wild boar (*Sus scrofa*) tissues

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Abstract

Due to the proximity of humans to the countryside and the progressive increase in populations of invasive species, such as wild boars (*Sus scrofa*), the risk of disease spread is also exacerbated, some of which are zoonoses caused by protozoa. In the present study, 75 tissue/organ samples from 25 wild boars obtained from authorized hunting in the northern region of Rio Grande do Sul were evaluated to investigate the presence of *Trypanosoma* spp. using conventional PCR with specific primers and amplification of the ITS1 region for *Leishmania* spp. detection and species differentiation, multiplex PCR with kDNA minicircle amplification was performed. *Trypanosoma* spp. DNA was detected in 11 out of 25 hearts, representing 44% of the culled animals. Regarding the detection of *Leishmania* DNA, *L. infantum* was detected in one spleen sample, accounting for 4%, and *L. amazonensis* in one liver sample from the same animal, also representing 4% (1/25) of the samples. It is important to note that this wild boar, with detection for both *L. amazonensis* and *L. infantum*, also had *Trypanosoma* spp. DNA detected in a heart sample, indicating the potential of this species to have multiple infections with these agents. Furthermore, this is the first reported case of multiple infection in a wild boar with these agents. Therefore, the results obtained reinforce the risk posed by invasive species, especially wild boars, as potential sources of infectious agent dissemination and their role as possible reservoirs for numerous diseases.

Keywords: Trypanosomiasis; Leishmaniasis; Molecular detection; Swine; One health; Poly-infection.

Introduction

In recent years, there has been an increase in the wild boar population in Brazil, mainly in Rio Grande do Sul, causing economic losses and damage to the local ecosystem (Ibama, 2020). According to Massei et al. (2015), the wild boar population has also been gradually increasing in other regions of the world, and such an increase can be considered a risk to human and animal health, since this invasive species is a potential reservoir and spreader of diseases (Miller et al. 2017). Therefore, wild boars (*Sus scrofa*) may be involved in the cycle and dissemination of several zoonoses and, due to their phylogenetic proximity to domestic pigs (*Sus scrofa domesticus*), they deserve increased attention regarding the transmission of microorganisms of importance in animal and human health. (Machado et al. 2019).

Some microorganisms are of great relevance, both for animal and human health, with *Trypanosoma* spp. and *Leishmania* spp. being considered parasites of reemerging diseases (Pedroso and Rocha 2009; Sinan 2008). According to the form of transmission, Trypanosomas are divided into the Stercoraria section and the Salivaria section, with the first being considered the contaminating form, which occurs through the vector's feces deposited near the bite. The second section acts in an inoculative way, through mechanical transmission during the bite (Hoare 1964).

Still within the Trypanosomatidae family, the transmission form in the genus *Leishmania* is by sandflies that become infected by biting infected hosts. The clinical presentations of the disease are cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL), and both forms can manifest in dogs and humans (Souza et al. 2014; Lonardoni et al. 2006). In Brazil, VL is endemic and important for public health, as it is a zoonosis, and the dog is the main reservoir of the parasite. However, wild animals may play a role in the *L. infantum* cycle (Dantas-Torres and Brandão-Filho 2006).

The proximity of humans and domestic animals to the field and, consequently, to wild animals increases the risk of transmission of zoonosis (Daszak et al. 2000). In view of this problem, the goal of this work is to detect DNA (Deoxyribonucleic acid) from *Trypanosoma* spp. and *Leishmania* spp. in tissues/organs of wild boars (*Sus scrofa*).

Material and methods

Samples of spleen, liver and heart were collected from wild boars (*Sus scrofa*) slaughtered in the northern region of Rio Grande do Sul in the shelter of the Wild Boar Population Control Program, in accordance with Normative Instruction 03 of January 31, 2013 (Brazilian Institute of the Environment - Ibama 2013), totaling 75 samples belonging to 25 animals, 15 males and 10 females. The material was classified by organ, stored in sterile

packages and transported under refrigeration to the Laboratory of Veterinary Microbiology and Immunology of the Instituto Federal Farroupilha (LAMIVET/IFFar), where they were stored at -20 °C. Portions were sent to the Parasite Diseases Laboratory of the Universidade Federal de Santa Maria (LADOPAR/UFSM) for molecular analysis.

DNA Extraction

The samples were submitted to the DNA extraction step, which was performed using the Wizard® Genomic DNA Purification commercial Kit from the company Promega®, using the manufacturer's instructions and adapted according to Bräunig et al. (2016). Until the time of analysis, DNA samples were stored at -20 °C.

Multiplex PCR *Leishmania* spp.

For the detection of DNA from species of *Leishmania* spp., the Multiplex PCR (polymerase chain reaction) designed by Conter et al. (2018) was performed. We used LSPF (5'GGGTAGGGGCGTTCTG3'), LVBR (5'GCGCGGCCCACTATA3'), LLAR (5'CCCCCAGTTGTGACCG3') and LLCR (5'CCGATTTTGAACGGGA3') primers in reactions with a final volume of 10 µL under the following conditions: 3 µL of total DNA (100 - 200 ng), 0.3 µL of MgCl2, 1 µL of 10x Taq buffer, 1 µL of Taq DNA polymerase (Thermo Fisher Scientific®), 0.2 µL of dNTPs (deoxyribonucleotides), 0.5 µL of each primer and 3.5 µL of ultrapure water; and subjected to the following thermocycling: initial denaturation for 3 minutes at 94 °C followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing for 90 seconds at 56 °C and extension at 72 °C for 15 seconds, with final extension for 10 minutes at 72 °C. The designed primers were based on the *Leishmania* kDNA minicircle region, amplifying 127 bp (base pairs) for the *Viannia* subgenus, 100 bp for *Leishmania amazonensis* and 60 bp for the *Leishmania donovani* and *Leishmania major* complex. PCR products were detected by 3% agarose gel electrophoresis, using molecular weight markers (Ladder 100 bp and 50 bp, Ludwig Biotec, Brazil®) and fluorescent dye (GelRed - Biotium, Hayward, CA, USA®). To visualize the fragments, the amplified products were submitted to ultraviolet light in a transilluminator. In all reactions, a positive control corresponding to each species evaluated was used, subgenus *Vianna*, *L. amazonensis* and complex *Leishmania donovani* / *Leishmania infantum* with DNA samples kindly provided by the Federal University of Pampa (Unipampa), Uruguaiana, Rio Grande do Sul, as Samples came from standard strains characterized by nucleotide sequencing, while Mili-Q Water was used as a negative control.

PCR *Trypanosoma* spp.

For identification of *Trypanosoma* spp., all PCR testings were performed in reaction volumes of 25 µL containing 2µL of total DNA [100 – 200 ng (nanograms)], 1.25 µL of MgCl2, 2.5 µL of 10x Taq, 1 U of Taq DNA

polymerase (0.2 µL) (Thermo Fisher Scientific®), 0.7 µL of dNTPs, 1 µM of each primer and 16.35 µL of ultrapure water. The positive control was composed of *Trypanosoma evansi* DNA, and ultrapure water was used as a negative control. The sequence of primers used were Kin1F (5'GCGTTCAAAGATTGGGCAAT3') and Kin2R (5'CGCCCGAAAGTTCACC3') according to Desquesnes et al. (2001), which amplifies the ITS1 gene, which is present in the conserved regions between 18S and 5.8S rDNA (ribosomal deoxyribonucleic acid), and its divergent products from 350 bp to 780 bp. The thermocycling conditions used were: initial denaturation at 94 ºC for 3 minutes, 35 cycles of denaturation at 94 ºC for 1 minute, annealing at 53 ºC for 1 minute and elongation at 72 ºC for 1 minute, and also 5 minutes at 72 ºC as the final step. The generated amplicons were detected by electrophoresis in a 1.5% agarose gel using molecular weight markers (Ladder 100 pb, Ludwig Biotec, Brazil®) and fluorescent dye (GelRed - Biotium, Hayward, CA, USA®) and, subsequently, visualization under ultraviolet light on a transilluminator.

Results and discussion

The results found are shown in Table 1, where it is possible to observe that the DNA of *Trypanosoma* spp. was detected in 11 of the 25 hearts, which represents 44% (11/25) of the animals slaughtered as carriers of this agent, figure 1 also shows the products obtained in the PCR reaction to detect *Trypanosoma* spp.. As for the detection of *Leishmania* DNA, the genetic material of *Leishmania infantum* was detected in 4% (1/25) of the samples, that is, in a spleen, and that of *Leishmania amazonensis* in a sample of the liver of the same animal, representing 4% (1/25), as shown in figure 2. This wild boar, which had *Leishmania* DNA detected in spleen and liver samples, also had *Trypanosoma* DNA detected in heart samples, indicating the potential of this species to present multiple infection with these agents.

Involved in the life cycle of these parasites are humans, the arthropod vector and many mammals that act as reservoirs. Peridomestic animals play an important role due to their close relationship with humans and triatomines, arthropod vectors of *Trypanosoma* spp., and also sandflies, arthropod vectors of *Leishmania spp*. In domestic pigs, for example, *Trypanosoma cruzi* has already been detected, characterizing their potential as a reservoir and possible role as a sentinel in surveillance and control programs as they are important maintainers of the parasite in nature (Herrera et al. 2008; Roque et al. 2008). Dogs remain as the main reservoir of *Leishmania* spp., despite this, the possibility of wild animals, such as wild boars playing an important role in the *Leishmania* cycle is not ruled out (Vidaletti et al. 2021).

The data found in this study revealing the positivity of wild mammals for the cited parasites corroborate with other studies carried out in Brazil, visually demonstrated in figure 3, indicating the occurrence of both agents in different regions of the country, with their detection together or individually. Herrera et al. (2008) demonstrated a similar rate (44.4%) of wild boars positive for *Trypanosoma* in Brazil and also agree with the data by Viettri et al. (2018), whose study showed positive wild boars (40%) for *Leishmania* spp. in Venezuela, both using the PCR technique. Still, other authors cite the detection of these agents using different detection techniques, such as blood culture and IFAT (Indirect Fluorescent Antibody Test) (Salazar-Schettino et al. 1997; Carraro 2020).

Porfirio et al. (2018) found anti-*T. cruzi*, anti-*T. evansi* and anti-*Leishmania* spp. in dogs and wild mammals infected with the same parasites, these findings demonstrate the risk of infection and the attention to the proximity of humans to these populations, in addition to cautioning that dogs can be reservoirs and sentinels for these diseases. In the central west and northeast regions of Brazil, Paula et al. (2010) and Ribeiro Jr et al. (2019) have shown the presence of *Trypanosoma* in vectors, as well as Costa et al. (2021) and Pita-Pereira et al. (2011) found positive vectors for *Leishmania* spp. in the north and south regions of the country. In addition to transmission by vectors, Herrera et al. (2011) highlights that the positivity of several wild mammals for *Trypanosoma* spp. may be related to the food web of these species, leading to possible trophic transmission. Besides pigs and wild boars, other domestic and wild species also showed *Trypanosoma* infection reported by authors in Brazil and in different countries, such as equine, bovine, caprine and ovine species (Araújo-Neto et al. 2023; Hassan-Kadle et al. 2020).

Through the data presented, it is possible to verify that the wild boar (*Sus scrofa*) participates in the wild cycle of *Trypanosoma* spp. and *Leishmania* spp., reinforcing the warning by Miller et al. (2017), which exposes the risk of infection to pathogens due to the presence of wild pigs concomitantly with humans and domestic animals. The growth of infectious exchanges between humans and wild animals can lead to new animal reservoirs, increasing unique health hazards (Thompson et al. 2009).

Conclusion

The detection of *Trypanosoma* spp. in 44% of heart samples, as well as *L. amazonensis* and *L. infantum* in the liver and spleen, underscores the risk posed by invasive species, particularly wild boars, as potential sources of infectious agent spread and their role as potential reservoirs for numerous diseases. Furthermore, this represents the first documented case of multiple infection in a wild boar involving *L. infantum*, *L. amazonensis* and *Trypanosoma* spp. Therefore, populations of invasive species must be controlled to prevent further infections and threats to both domestic animal and human populations.

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Author Contributions

Bibiana Rodrigues de Freitas: Conceptualization, Methodology, Data curation, Writing- Original draft. Gilneia da Rosa: Investigation, Data curation. Isac Junior Roman: Methodology, Data curation, Investigation. Letícia Trevisan Gressler: Investigation, Data curation. Juliana Felipetto Cargnelutti: Investigation, Data curation. Fernanda Silveira Flôres Vogel: Data curation, Supervision, Visualization, Investigation, Writing—review and editing. Validation. Rodrigo Casquero Cunha: Supervision, Visualization, Writing—review and editing.

Data availability

Not applicable.

Ethics approval

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with samples received for diagnosis.

Consent to participate

Not applicable.

Consent for publication

All authors consent to publication of this manuscript.

Conflict of interest

The authors declare no competing interests.

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Table 1 Frequency distribution of PCR reaction results for molecular detection of *Trypanosoma* spp. and *Leishmania* spp. in organs of wild boars (*Sus scrofa*).

Figura 1 PCR *Trypanosoma* spp. Electrophoresis in 1.5% agarose gel showing PCR products using the primers Kin1F/Kin2R rRNA ITS1 gene, from wild boar tissue samples. Lane 1: 100 bp ladder size marker; lane 2: negative control - no DNA; lane 3 positive control *Trypanosoma evansi* DNA with expected product of 540 bp; lane 4 to lane 8 heart samples positive for *Trypanosoma* spp.; lane 9 and 10 samples tested negative by PCR.

Figure 2 Diagnosis of leishmaniasis Multiplex PCR. 3% agarose gel electrophoresis showing PCR products using the primers LSPF/LVBR/ LLAR *Leishmania* kDNA minicircle region, from wild boar tissue samples. Lane 1: 50 bp ladder size marker; lane 2: negative control - no DNA; lane 3 positive controls DNA *Viannia* subgenus 127 bp, *Leishmania amazonensis* 100 bp and *Leishmania donovani* and *Leishmania major* complex 60 bp; lane 4 positive spleen sample for *Leishmania infantum* 60 bp; lane 5 positive liver sample for *Leishmania amazonensis* 100 bp.

Figure 3 Brazil's map. In orange states with detection described for *Tryapnsoma* spp in feral pigs, in purple regions with detection for *Leishmania* spp and *Tryapnsoma* spp., and in brown detection of only *Leishmania* spp. Particularly noteworthy is the state of Rio Grande do Sul, which includes the current study detecting both agents in tissues from wild boars slaughtered in the northern region of the state.

Highlights

- ✓ Animals considered exotic such as the Wild Boar (*Sus scrofa*) can be disseminators of zoonotic diseases.
- ✓ DNA from *Trypanosoma* spp. was detected in boar hearts.
- ✓ In liver and spleen samples it was possible to identify DNA from *L. infantum* and *L. amazonensis*.
- ✓ It is the first report of polyinfection with *Trypanosoma* spp., *L. infantum* and *L. amazonensis* in wild boars.

Figure 1

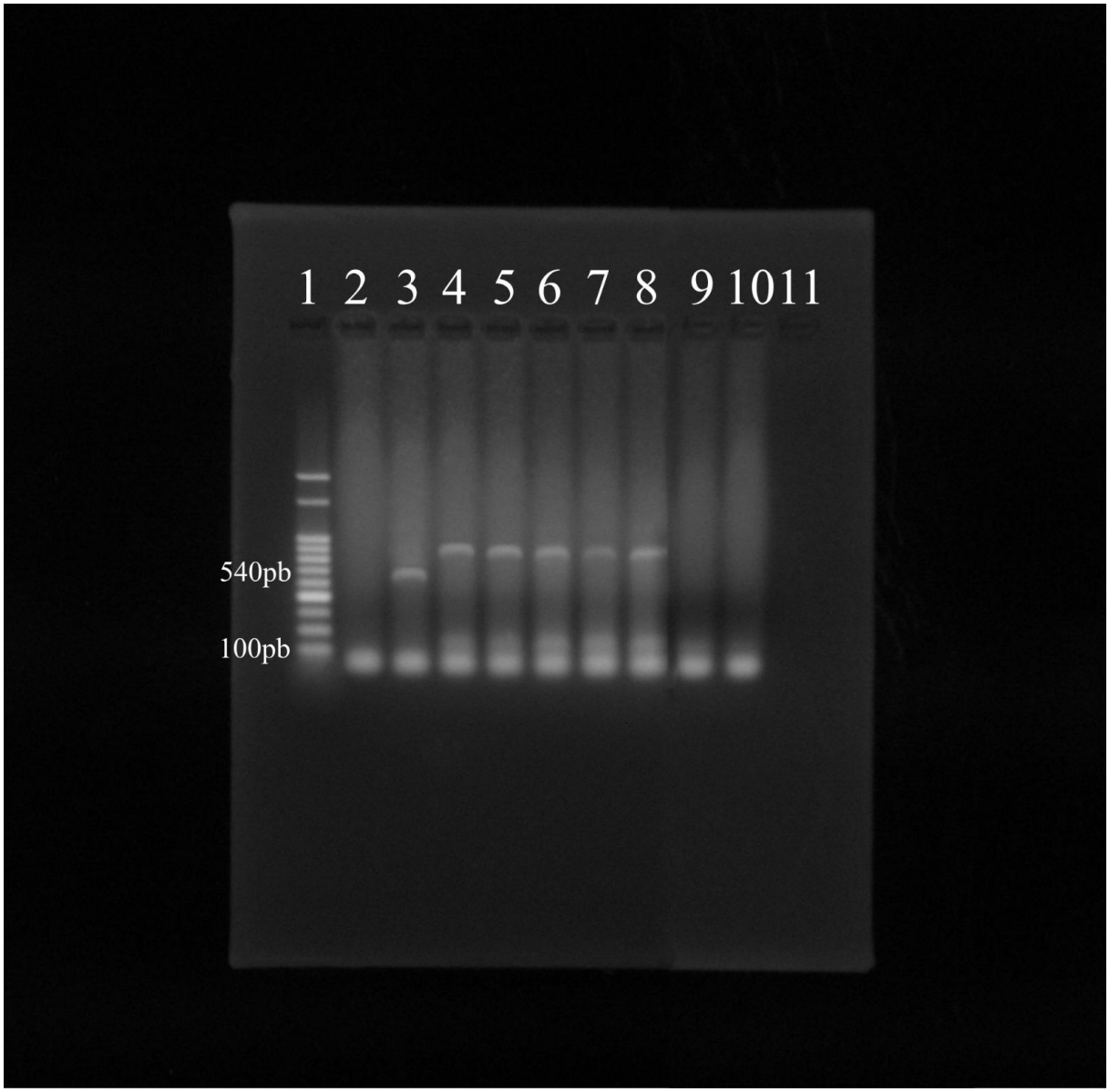

Figure 2

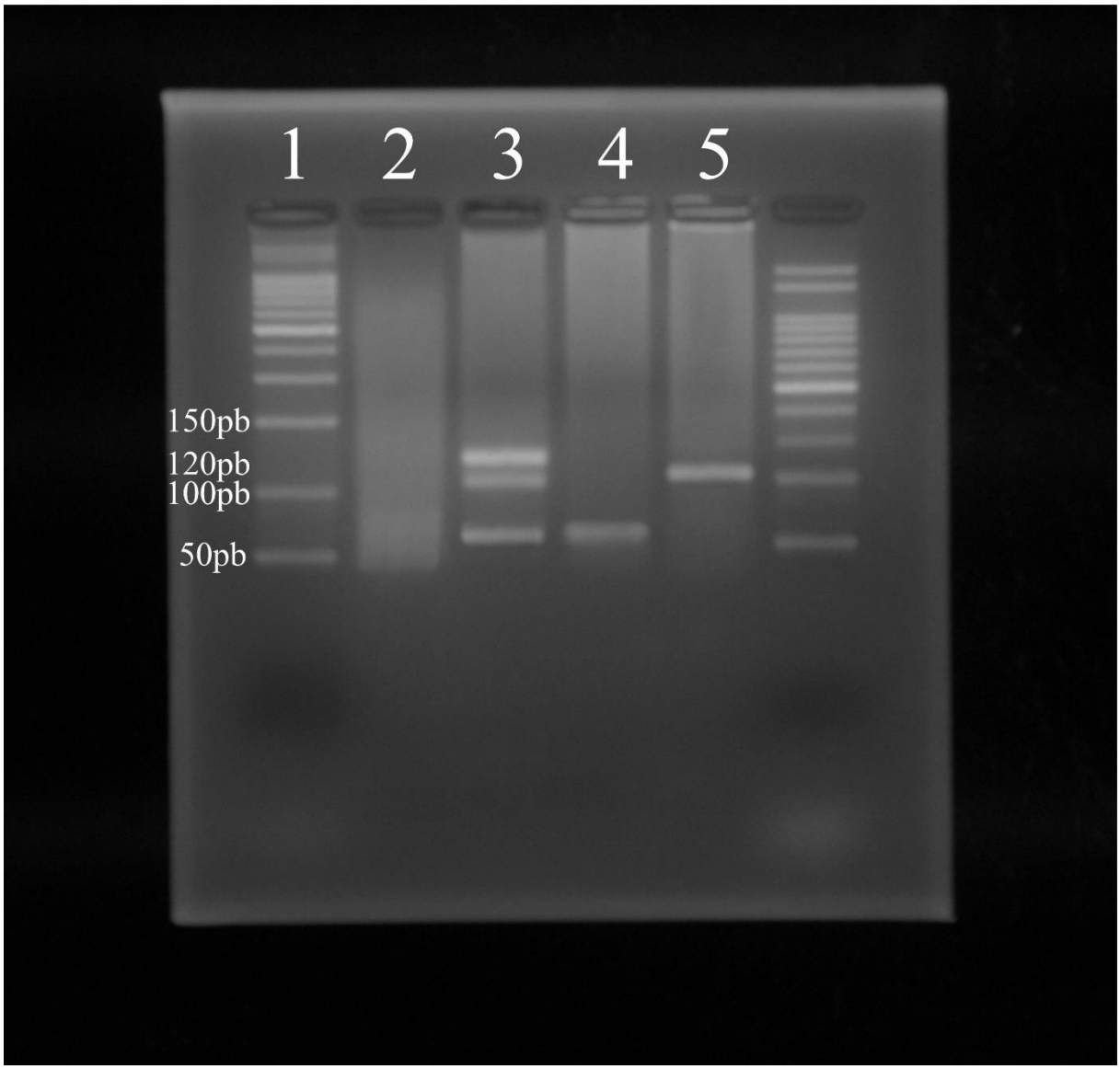

Figure 3

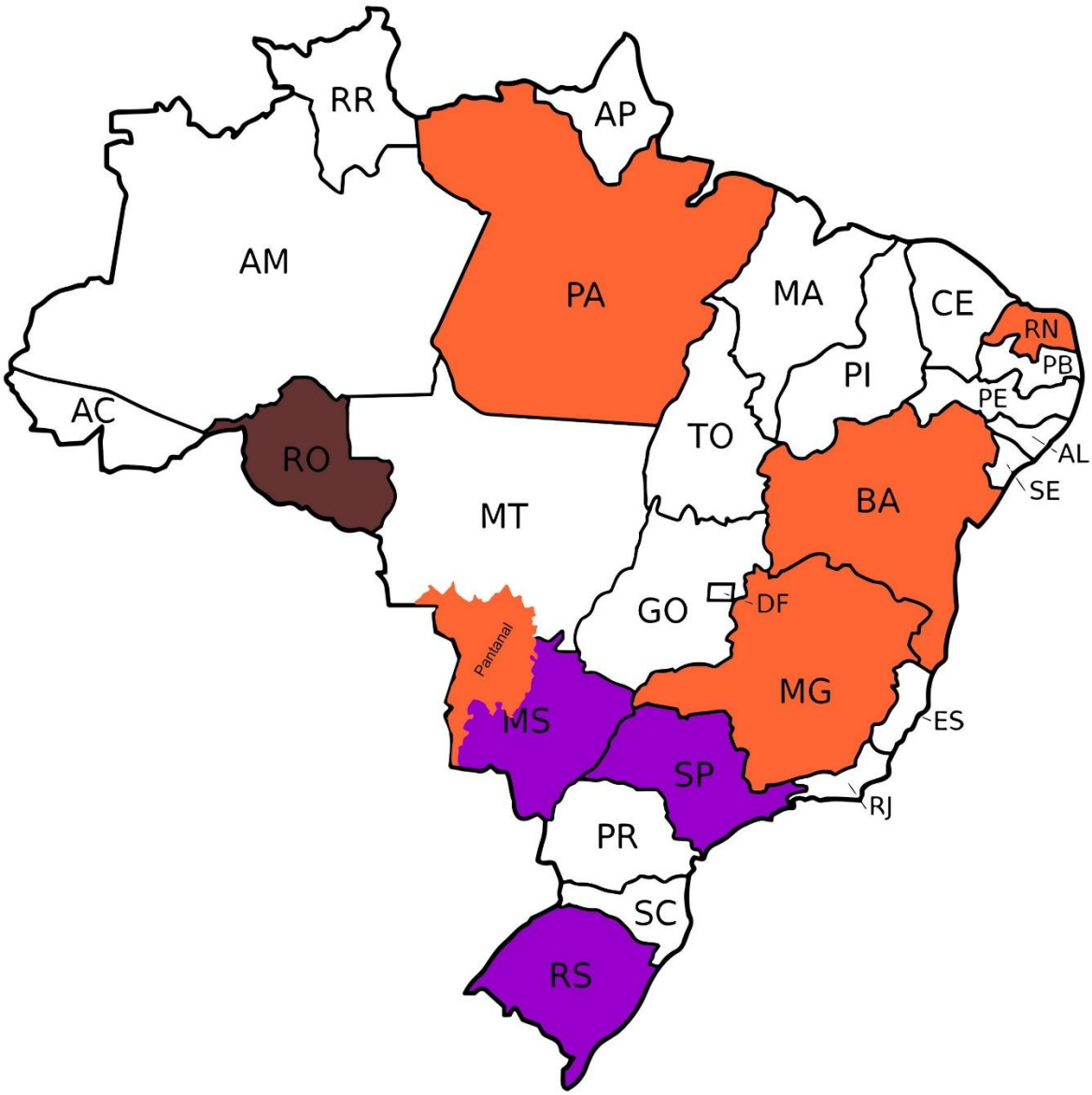
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Table 1 Frequency distribution of PCR reaction results for molecular detection of *Trypanosoma* spp. and *Leishmania* spp. in organs of wild boars (*Sus scrofa*).

	***Trypanosoma* spp.**	***Leishmania infantum***	***Leishmania amazonensis***
Heart (n=25)	11 (44%)	-	-
Spleen (n=25)	-	1 (4%)	Negative
Liver (n=25)	-	Negative	1 (4%)

Data are expressed by the number of positive samples followed by the percentage of positivity.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential
competing interests.

Ethical Statement

Ethical Approval: No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with samples received to analyses routine.

4 Considerações Finais

O javali pode ser considerado um disseminador de agentes zoonóticos como *Trypanosoma* spp. e *Leishmania* spp.. O aumento da população de javalis, a competição entre espécies, os prejuízos aos biomas locais e a proximidade dos porcos selvagens com o ser humano, são fatores que podem aumentar a chance de infecção de animais e pessoas.

Dados que demonstram a presença do DNA de *Trypanosoma* spp. e *Leishmania* spp. em javalis (*Sus scrofa*), auxiliam na elucidação de questões acerca da participação de animais silvestres e exóticos nos ciclos silvestres dos protozoários citados. Assim como estudos como este reforçam a importância das políticas de controle de populações consideradas invasoras, visto seus prejuízos financeiros e sanitários à pecuária e à população.

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