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DETECÇÃO E CARACTERIZAÇÃO DE VÍRUS ENTÉRICOS EM ROEDORES CAVÍDEOS E QUIRÓPTEROS MOLOSSÍDEOS, FILOSTOMÍDEOS E VESPERTILIONÍDEOS

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Linha de Pesquisa: Desenvolvimento em Virologia

Orientador: Prof. Dr. Matheus Nunes Weber
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Dissertação apresentada para obtenção do grau de mestre em Virologia pela Universidade Feevale.

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SUMÁRIO

RESUMO

O Brasil possui dimensões continentais e uma imensa biodiversidade, com o maior número de espécies animais endêmicas em escala global. A invasão de ecossistemas naturais facilita o contato entre pessoas, animais domésticos e silvestres, favorecendo o intercâmbio de agentes virais. Sabe-se que roedores e quirópteros desempenham um papel crucial na transmissão de vários vírus, destacando-se o fato de atuar como reservatórios de agentes virais de grande importância na saúde única, como por exemplo membros das famílias *Coronaviridae, Paramyxoviridae*, *Filoviridae, Hantaviridae* e *Arenaviridae*. Além disso, muitas espécies são sinantrópicas, coabitando determinados espaços com seres humanos. A ecovigilância em espécies animais da ordem Rodentia e Chiroptera são extremamente relevantes para a saúde única. Portanto, este trabalho objetivou pesquisar, identificar e caracterizar vírus entéricos em roedores e morcegos em áreas urbanas, periurbanas e rurais. Desta forma, foi dividido em dois capítulos: No primeiro, buscou-se a pesquisa e caracterização de membros da família *Adenoviridae* em preás (*Cavia aperea aperea*) da região do sertão da Paraíba. No segundo, avaliou-se a presença de adenovírus e rotavírus em morcegos molossídeos, filostomídeos e vespertilionídeos capturados no Rio Grande do Sul no período de setembro de 2021 a julho de 2022. No primeiro capítulo, foram analisadas 14 amostras de suabes retais de preás por nested-PCR para detecção de adenovírus, onde duas amostras foram positivas e caracterizadas como mastadenovíus humano C (HAdV-C) após sequenciamento e análise filogenética. No segundo capítulo, foram analisadas 60 amostras de suabes retais por nested-PCR e RT-PCR para detecção de adenovírus e rotavírus, respectivamente. Todas as amostras testaram negativo para rotavírus e 13,3% (8/60) testaram positivo para adenovírus, sendo três classificadas como HAdV-C e cinco como HAdV-E. Desta forma, o presente estudo auxiliará na ecovigilância quanto a agentes virais geneticamente relacionados a patógenos de importância na saúde única.

Palavras-chave: Mastadenovíus; Rotavírus; Sequenciamento; Ecovigilância; Saúde única.

ABSTRACT

Brazil has continental dimensions and immense biodiversity, with the largest number of endemic animal species on a global scale. The invasion of natural ecosystems facilitates contact between people, domestic and wild animals, favoring the exchange of viral agents. Rodents and chiropters are known to play a crucial role in the transmission of several viruses, highlighting the fact that they act as reservoirs of viral agents of great importance in One Health, such as members of the *Coronaviridae, Paramyxoviridae, Filoviridae, Hantaviridae,* and *Arenaviridae* families. In addition, many species are synanthropic, cohabiting certain spaces with humans. Ecovigilance in animal species of the order Rodentia and Chiroptera are extremely relevant to the One Health. Therefore, this work aimed to research, identify and characterize enteric viruses in rodents and bats in urban, peri-urban and rural areas. Thus, it was divided into two chapters: In the first, we sought the research and characterization of members of the *Adenoviridae* family in wild guinea pigs (*Cavia aperea aperea*) from Paraíba state. In the other one, we evaluated the presence of adenovirus and rotavirus in molossid, philostomid and vespertilionid bats captured in Rio Grande do Sul state from September 2021 to July 2022. In the first chapter, we analyzed 14 samples of rectal suabes from prey by nested-PCR for adenovirus detection, where two samples were positive and characterized as human mastadenovirus C (HAdV-C) after sequencing followed by phylogenetic analysis. In the second chapter, 60 rectal swab samples were analyzed by nested-PCR and RT-PCR for detection of adenovirus and rotavirus, respectively. All samples tested negative for rotavirus and 13.3% (8/60) tested positive for adenovirus, with three classified as HAdV-C and five as HAdV-E. Thus, the present study will assist in ecovigilance for viral agents genetically related to pathogens of One Health importance.

Keywords: Mastadenovirus; Rotavirus; Sequencing; Ecovigilance; One Health.

1. INTRODUÇÃO

O fato que muitos animais podem ser reservatórios de agentes potencialmente patogênicos, torna importante seu monitoramento. Muitos fatores podem contribuir para a emergência de novos patógenos, como atividades antrópicas, mudanças climáticas e ambientais (BORREMANS et al., 2019). A emergência de agentes virais recentemente reportados, como o coronavírus causador da COVID-19 e o *monkeypox*, reforçam esses fatos (UMAKANTHAN et al., 2020; BUNGE et al., 2022).

O Brasil possui cerca de 751 espécies de mamíferos silvestres catalogadas, distribuídas em 249 gêneros, 51 famílias e 11 ordens, sendo que 267 espécies são roedores e 182 de morcegos (QUINTELA; DA ROSA; FEIJÓ, 2020). A ordem Rodentia constitui o grupo de mamíferos placentários mais diversificados, onde representam 42% das espécies conhecidas e sendo provavelmente a maior ordem dos mamíferos, com alto potencial no processo de propagação das doenças transmissíveis em populações animais (CARVALHO, 2011). No Brasil, os roedores representam cerca 34% de todos os mamíferos, compreendendo 267 espécies divididas em nove famílias, contudo, revisões taxonômicas estão em constante mudança devido ao aprimoramento na metodologia filogenética, técnicas moleculares e maior acesso a esses dados (PAGLIA et al., 2012; ABREU et al., 2022). Os roedores silvestres resistem ameaças e desafios com destruição dos seus habitats, fragmentação das florestas, exploração dos recursos naturais e industrialização da agricultura. Esses distúrbios ambientais vêm favorecendo o aumento do surgimento de zoonoses emergentes, contribuindo para disseminação de doenças entre humanos e outros animais. (CARVALHO, 2011; LACHER et al. 2020). Os roedores são responsáveis por diversos ciclos epidemiológicos que resultam em importantes doenças nos seres humanos e outros animais, como equinococose alveolar, hantaviroses e leptospirose. Diferentes vírus já foram isolados e detectados em roedores, com importância para vírus das famílias *Arenaviridae*, *Bunyaviridae*, *Hantaviridae*, *Coronaviridae* e *Poxviridae* (LACK et al., 2012; ZAPATA et al., 2013; DE OLIVEIRA et al., 2014).

A ordem Chiroptera representa a segunda ordem de maior riqueza no Rio Grande do Sul, superada somente pela Rodentia (WEBER; ROMAN; CACEREZ, 2013). Os morcegos, por pressões evolutivas, são os únicos mamíferos voadores, possuem ampla distribuição mundial, com exceção dos polos (NOWAK, 1999). No Rio Grande do Sul estão registradas 40 espécies de morcegos distribuídas em quatro famílias com quinze

gêneros (BERNARDI et al., 2007; PASSOS et al., 2010). Na última década, morcegos têm sido identificados como reservatórios naturais de vários vírus emergentes, causando surtos de doenças relevantes para a saúde pública como Ebola, Hendra, Nipah, a síndrome respiratória aguda grave (SARS) causada pelo SARS-CoV, a Síndrome Respiratória do Oriente Médio (MERS) e a doença atualmente pandêmica do Coronavirus 2019 (COVID-19), causada pelo SARS-CoV- 2 (DREXLER et al., 2012; MONCHATRE et al., 2017; CIBULSKI et al., 2020). Outros subtipos emergentes do vírus influenza A, com hemaglutininas e neuraminidades previamente não descritas, também têm sido reportados em quirópteros, estabelecendo-os como importantes reservatórios, indicando uma ampla circulação desses vírus em populações de morcegos (TONG et al., 2013). Cabe ressaltar que até o momento esses influeza vírus A com hemaglutininas e neuraminidades somente reportadas em vírus detectados em morcegos não apresentam indícios de infecção em seres humanos (CIMINSKI et al., 2019). Contudo, para esta categoria de agentes virais, eventos de recombinação homóloga e ressortimento não podem ser descartados.

Devido ao grande número de espécies de morcegos presentes no País, há um crescente interesse e necessidade da investigação de agentes virais relacionados a patógenos humanos. Os morcegos constituem uma grande diversidade de espécies, apresentando um dos grupos de mamíferos mais diversificados quanto aos hábitos alimentares e exploram praticamente todos os níveis tróficos, exceto saprófagos, aumentando a possibilidade de contato com humanos e animais domésticos (DOS REIS et al., 2007).

O aumento da ocorrência das doenças infecciosas emergentes pode estar relacionado diretamente com a ação da população humana, baseado na presunção que mudanças amplamente antropogênicas, como a expansão da agricultura, comércio, rotas de viagem e consumo de animais silvestres, favoreceram a destruição de *hotspots* e com isso, possíveis surgimentos de patógenos zoonóticos emergentes (MORSE et al., 2012).

Muitas espécies de roedores e morcegos silvestres apresentam uma relação de sinantropia com ambiente urbano, uma relação de convivência e proximidade as habitações humanas. Além disso, apresentam adaptações variadas à urbanização, algumas capazes de explorar construções feitas pelo homem e ajustar às novas condições ambientais com a possibilidade de transmissões de doenças infecciosas para animais domésticos e humanos. (ROQUE; JANSEN, 2014; TZORTZAKAKI et al., 2019). Os roedores e morcegos sinantrópicos são reservatórios de uma grande diversidade de vírus zoonóticos, com potencial de transmissão, conseguindo infectar ou coinfectar novos

hospedeiros (KOHL; NITSCHE; KURTH, 2021; TORRES-CASTRO, 2017). Na Itália morcegos pertencentes diferentes famílias foram positivos para adenovírus, considerados uma possível ameaça zoonótica para os seres humanos e outros animais (DIAKOUDI et al., 2019). Muitos adenovírus humanos (HAdVs) têm sido cada vez mais reconhecidos como principais contribuintes para doenças que variam de assintomáticas a fatais, com possíveis transmissões zoonóticas, podendo atravessar a barreira entre diferentes espécies (MEDKOUR et al., 2020). Os adenovírus pertencentes ao gênero *Mastadenovirus* infectam uma ampla gama de hospedeiros mamíferos, incluindo roedores, morcegos, bovinos, caninos, veados, golfinhos, equinos, primatas não humanos, ovinos, suínos, leões marinhos, gambás, esquilos e musaranho-arborícola (BORKENHAGEN et al., 2019).

Outro agente infeccioso de interesse à Saúde Pública são os rotavírus, responsáveis por causar doenças diarreicas agudas em mamíferos e aves (VLASOVA; AMIMO; SAIF, 2017). As infecções por rotavírus também são descritas em morcegos, onde os autores descreveram seis novos genótipos, com similaridade genéticas a outros rotavírus encontradas em outros mamíferos e quirópteros (SIMSEK et al. 2021). Em Bangladesh, um estudo relatou a presença de rotavírus em roedores das espécies *Mus musculus* e *Rattus rattus* (ISLAM et al., 2022).

Com isso, o presente estudo busca a identificação de vírus entéricos relacionados a patógenos humanos em animais das ordens Rodentia e Chiroptera, a fim de ampliar o conhecimento acerca da riqueza viral encontrada nessas ordens. Os dados aqui gerados serão de extrema importância para ecovigilância.

2. OBJETIVOS

2.1 Objetivo geral

Pesquisar, identificar e caracterizar vírus entéricos em roedores (cavídeos) e morcegos (molossídeos, filostomídeos e vespertilionídeos) em áreas urbanas, periurbanas e rurais.

2.2 Objetivos específicos

I. Pesquisar por PCR vírus da família *Adenoviridae* em roedores da espécie *Cavia aperea aperea*;

II. Pesquisar por PCR e RT-PCR vírus das famílias *Adenoviridae* e *Reoviridae* em amostras de suabes retais de quirópteros;

III. Sequenciar os produtos de amplificação;

IV. Determinar as espécies de roedores e morcegos que podem carrear esses vírus.

3. CAPÍTULO 1 – DETECÇÃO DE MASTADENOVÍRUS HUMANO C EM FEZES DE PREÁS (*Cavia aperea aperea*)

Os resultados obtidos nos experimentos que caracterizam o capítulo 1 serão apresentados a seguir sob a forma de artigo científico, tendo sido submetido e publicado no periódico *Brazilian Journal of Microbiology* (fator de impacto 2,214).

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ENVIRONMENTAL MICROBIOLOGY - SHORT COMMUNICATION

Detection of human *Mastadenovirus* C in wild guinea pigs (*Cavia aperea aperea*) feces

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Abstract
The Adenoviridae family is composed by a high diversity of viruses that are extremely resistant in environment and are frequently excreted in animal reservoir feces for long periods. The knowledge of adenovirus (AdV) diversity among wild species may be important for the understanding of the epidemiology of putative emerging diseases. *Cavia aperea aperea*, commonly known as wild guinea pigs, wild cavies, or preas, are small herbivorous rodents widely distributed throughout South America and classified in Caviidae family, as well as domestic guinea pigs and capybaras. In order to investigate their potential role as reservoir of zoonotic agents, the present study aimed to verify the presence of AdV in fecal samples of 14 preas from Northeast Brazil. When submitted to nested PCR, two out of 14 samples (14.28%) were positive for AdV and classified as human *Mastadenovirus* C (HAdV-C) using DNA sequencing and phylogenetic analysis. Wild guinea pigs are synanthropic rodents that live in close contact with humans. The investigation of viral agents in rodents is important due to their potential role as reservoirs of human and animal pathogens. Moreover, the present work presents the first known evidence of HAdV in wild guinea pig stool samples, which may represent both the impact of anthropogenic pollution to wild animals and an important knowledge in terms of human health.

Keywords *Adenoviridae* · Prea · HAdV-C · Rodents · Wild cavy

Introduction

The order Rodentia constitute the most diverse order of mammals [30], currently covering ~ 258 species in Brazil [26]. Wild guinea pigs (*Cavia aperea aperea*), commonly known as preas or wild cavies, are rodents belonging to the Caviidae family, as well as guinea pigs (*Cavia aperea porcellus*) and capybaras (*Hydrochoerus* sp.). They are widely distributed across South America, presenting social behavior and morning and nocturnal scavenger habits, besides raised as food in some South American regions [25]. Some authors consider the wild guinea pig the wild ancestor of the guinea pig, which is reared as a domestic and laboratory animal [2, 4, 16]. These rodents can harbor zoonotic pathogens, such as *Leptospira* sp., *Salmonella typhi*, *Trypanosoma cruzi*, *Giardia* sp., and *Cryptosporidium* sp. [13, 21]. In addition, rodents are known to act as reservoirs of zoonotic agents extremely serious to human health, such as hantaviruses and arenaviruses [20, 24].

Enteric infections can contribute to major causes of morbidity and mortality worldwide and may result in the contamination of environment, animals, water, and food. In developing countries, which have less access to basic sanitation services, these infections can present a serious threat to human and animal health [29]. Enteric viruses are present in the digestive system of susceptible individuals, carriers, and reservoirs [31].

The Adenoviridae family contains some members that are important cause of gastrointestinal disease in humans and animals [12]. Adenoviruses are non-enveloped

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viruses constituted of double-stranded, linear DNA viruses of ~ 34–36 kbp in length. They are grouped in six genera, where the *Mastadenovirus* genus presents 51 species that may infect a large number of mammals [15]. There are 88 known genotypes of human mastadenoviruses (HAdVs) grouped into seven species classified as A through G. Agents classified in the HAdV-D species present the majority of genotypes, consisting of 57 types, followed by the HAdV-B species with 16 types [7]. The HAdVs may be associated with a wide variety of diseases and can lead to tropism in different organs resulting to clinical signs in humans, which include acute conjunctivitis, cystitis, acute febrile pharyngitis, respiratory, and systemic infections in immunosuppressed individuals. The HAdV-A, B, C, and E species may cause a range of clinical diseases, leading to gastrointestinal and respiratory infections, while the HAdV-D, F, and G species are more commonly associated with disorders in digestive system. HAdV-B, D, and E may be also associated with ocular diseases [11].

Increasing urbanization which added deforestation and decrease in vegetation cover providing greater contact between wild animals and humans [23]. Moreover, the greater contact between humans and wild animals may be mainly linked to the absence of refuges, decrease in food resources, and adaptations to urbanized areas [9]. Wild or exotic animal species that have the ability to adapt in urban areas and share the same niche temporarily or even permanently in these places are identified as synanthropic [3]. Synanthropic rodents play an important role in the dissemination and maintenance of several infectious agents responsible for numerous zoonotic infections of national and global importance, participating as reservoirs or intermediate hosts [33]. Thus, the present study verified the presence of adenoviruses in fecal samples of *Cavia aperea aperea* obtained from animals in the northeast region of Brazil.

Materials and methods

In May 2020, rectal swab samples were obtained of 14 apparently healthy free-ranging *Cavia aperea aperea* within a 3-week interval in the municipality of Brejó do Cruz, Paraíba state (Northeast Brazil), using trap-door traps. The traps were placed in a transition region between the urban and rural areas in the semi-arid, with observation of the traps twice a day (early morning and late afternoon). The animals were captured, collected, and immediately released at the same site. The samples were conditioned in 20% (w/v) phosphate-buffered saline (PBS, pH 7.4) and stored at − 80 °C for further analysis. The project was registered with the Ethics Committee on Animal Use (CEUA) of the Federal University of Campina Grande (UFCG) under the protocol number no. 023/2017.

Total DNA was isolated using MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific™) in an automatic KingFisher™ Duo Prime (Thermo Fisher Scientific™) of a total volume of 200 µL of rectal swab sample and eluted in 90 µL of ultrapure water. A nested PCR was performed in order to amplify a 261-bp fragment of AdV polymerase (Table S1) [17]. A HAdV-5 positive sample obtained from the Laboratory of Molecular Microbiology at Feevale University was used as positive control, while ultrapure water was used as a negative control.

The positive amplification products were purified using the PureLink™ PCR Purification Kit (Thermo Fisher Scientific) and had quantity and quality analyzed by fluorometry using QuBit and Nanodrop, respectively. The sequencing of the samples was performed at the ACTGene Laboratory (Biotechnology Center, UFRGS, Porto Alegre, RS) using the ABI-PRISM 3100 Genetic Analyzer automated sequencer armed with 50 cm capillaries and POP6 polymer (Applied Biosystems, USA). The template DNAs (30 to 60 ng) were labeled using 3.2 pmol of the primer 5′-NNNNNNNNNNNNNNN-3′ and 2 µL of BigDye Terminator v3.1 Cycle Sequencing RR-100 reagent (Applied Biosystems) in a final volume of 10 µL. The labeling reactions were performed on a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) with an initial denaturation step at 96 °C for 3 min followed by 25 cycles of 96 °C for 10 s, 55 °C for 5 s, and 60 °C for 4 min. After labeling, the samples were purified by precipitation with isopropanol and washing with 70% ethanol. The precipitated products were diluted in 10 µL formamide, denatured at 95 °C for 5 min, cooled on ice for 5 min, and electro injected into the automated sequencer. Sequencing data were collected using data collection v1.0.1 software (Applied Biosystems) with parameters Dye Set "Z," Mobility File "DT3100POP6{BDv3}v1.mob," BioLIMS Project "3100_Project1," Run Module 1 "StdSeq50_POP6_50_cm_cfv_100," and Analysis Module 1 "BC-3100SR_Seq_FASTA.saz." The positive control was also submitted for DNA sequencing in order to discharge contamination.

Sequence editing and de novo assembly were performed using the Geneious Prime 2022.1 bioinformatics suite. The sequences were submitted for nucleotide BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). After, nucleotide sequences were aligned using the MAFFT software [27]. Phylogenetic tree was constructed using maximum likelihood (ML) inference and GTR + G + I substitution model with the program MEGA6 program [32]. The robustness of the hypothesis was tested in 1000 replicates.

Results and discussion

In the present study, rectal swab samples of *Cavia aperea aperea* were analyzed by nested PCR [17], where 14.28% (2/14) tested positive. The positive samples were sampled in the same location but within a 10-day interval. The positive amplification products were submitted for DNA sequencing. The sequences were analyzed using the nucleotide BLAST, resulting in 98.85% identity with HAdV-C strain 46C6 (GenBank accession number MH121113.1) detected in human fecal samples in Germany. The two positive samples obtained in the present study showed 100% identity between each other and 98.47% nucleotide identity when compared to the positive control used which discharged putative cross-contamination. In partial polymerase AdV fragment phylogenetic analysis, using representative sequences of HAdV-A to G and animal AdV (Fig. 1), bootstrap values of 92 to 100% at the nodes delimiting the species clusters were observed reinforcing the robustness of the hypothesis. The samples detected in the present study clustered in the HAdV-C clade closely related to HAdV-C detected in Germany, USA, China, and Singapore. HAdV-C may cause gastrointestinal and respiratory infections in humans [11] and are generally more clinically relevant than HAdV-B, E, and D in immunocompromised patients [1, 10, 18].

The detection of the viral genome in the feces of these animals may be possible ingestion of contaminated water and food and excreted in high fecal concentrations into the environment [5]. Some studies reported HAdV-C detection in fecal samples from wild carnivores such as free-living gray pampean foxes (*Lycalopex gymnocercus*) and crab-eating foxes (*Cerdocyon thous*), where 14 of the 17 samples analyzed were contaminated, demonstrating possible contamination of the animals by ingesting human waste [22]. Our results demonstrate the complexity of the viral infections that these animals may be susceptible to and may assist in putative reservoirs monitoring, contributing to health surveillance. These data point to the influence of anthropogenic growth on the natural habitats of wild animals. Wild guinea pigs are synanthropic rodents that live in close contact with humans. In addition, they are bred in captivity for human consumption in some regions in South America [28]. The research of viral agents in rodents is important since they can be reservoirs of human and animal pathogens [13, 21], such as hantavirus and arenavirus [20, 24]. Moreover, the present study evidences the first detection of HAdV in wild

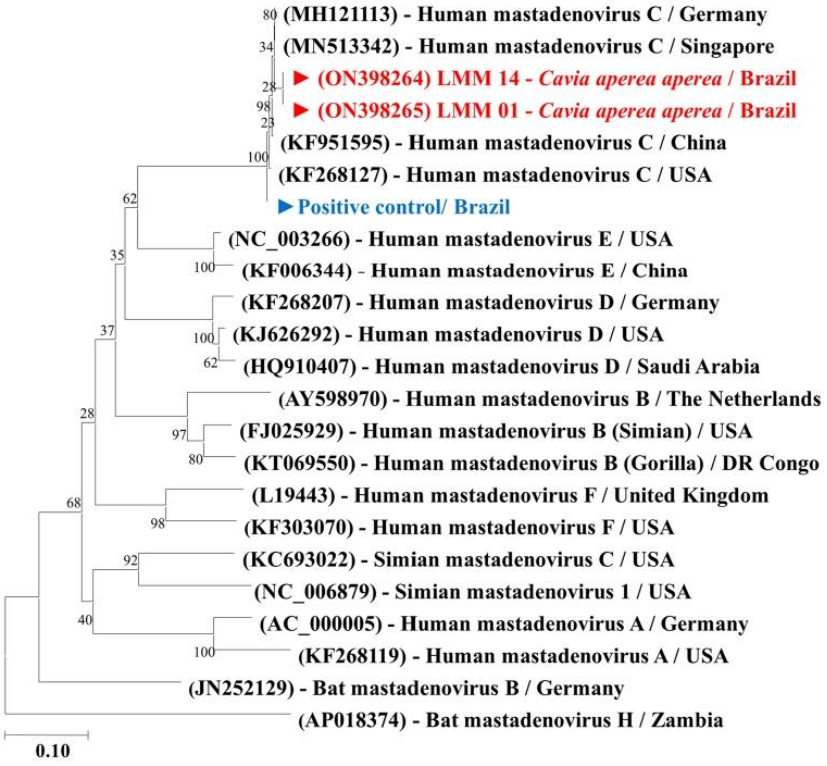

Fig. 1 Nucleotide phylogenetic tree constructed using adenovirus polymerase gene. The phylogenetic tree was constructed using MEGA 6 software using the maximum likelihood algorithm method based on the GTR + G + I model in 1000 replicates. The two positive samples detected in the present study, in addition with the positive control used in nested PCR, are highlighted with a ► symbol

guinea pig stool samples, which may represent an important knowledge for human health.

The presence of HAdV-C was recently reported in a previous study, which analyzed white-eared opossums (*Didelphis albiventris*) feces, suggesting exposure of these animals with contaminated water or food [19]. Other study reported the detection of adenovirus in fecal samples of South American fur seals (*Arctocephalus australis*), where the obtained nucleotide sequences showed a great similarity with HAdV-C [6]. The presence of HAdV-C was also reported in stool samples of frugivorous bats (*Sturnira lilium*) [8]. The spread of enteric viruses by animals may be a potential contamination source for environment and aquatic ecosystems, where these viral agents have been considered fundamental as indicators of water quality and health risks that may harm human and animal health [14]. Our work expand the spectrum of putative wild reservoirs of HAdV-C.

Conclusions

In the present study, we reported the presence of HAdV-C in wild guinea pig feces using nested PCR, DNA sequencing, and phylogenetic analysis. These findings increase the knowledge about the viral community present in these wild animals and suggest a putative potential as HAdV reservoir, likewise bringing important information on the genetic diversity and geographical distribution of these viruses to anticipate prevention and control measures of zoonotic diseases.

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Declarations

Conflict of interest The authors declare no competing interests.

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4. CAPÍTULO 2 – AVALIAÇÃO DA PRESENÇA DE ADENOVÍRUS E ROTAVÍRUS EM QUIRÓPTEROS MOLOSSÍDEOS, FILOSTOMÍDEOS E VESPERTILIONÍDEOS CAPTURADOS NO RIO GRANDE DO SUL, BRASIL

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Evaluation of adenovirus and rotavirus presence in molossid, phyllostomid, and vespertilionid bats captured in Rio Grande do Sul, Southern Brazil

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Abstract

Bat-borne viruses significantly may affect public health and the global economy. These mammals present a wide geographical distribution, and unique biological, physiological, and immunogenic characteristics, allowing the dissemination of many known and unknown viruses. Enteric viruses as adeno (AdV) and rotaviruses are recognized as main causative agents of disease and outbreaks. In the present study, the presence of viruses from *Adenoviridae* and *Reoviridae* families was evaluated in molossid, phyllostomid, and vespertilionid bats captured in Rio Grande do Sul, Southern Brazil, between September 2021 to July 2022. Sixty bat rectal swabs were analyzed by PCR. Eight (13.3%) samples were positive for adenovirus and classified as human mastadenovirus C (HAdV-C) (three samples) and HAdV-E (five samples) by sequencing followed phylogenetic analysis. All samples were negative in rotavirus specific RT-PCR. This is the first study to describe the presence of HAdV in samples of *Glossophaga soricina*, *Eptesicus brasiliensis*, and *Histiotus velatus*. Furthermore, the presence of HAdV-E in bats was reported, which is unusual and may suggest that other HAdV genotypes, in addition to HAdV-C, may also be harbored by wild animals. The data generated in the present study reinforces the importance of eco-surveillance of viral agents related to diseases in humans and wild animals. In addition, it is essential to identify possible new hosts or reservoirs that increase the risk of spillover and dissemination of infectious pathogens, helping to prevent and control zoonotic diseases.

Keywords: Chiropterans, *Adenoviridae*, *Reoviridae*, eco-surveillance, One health.

Introduction

Enteric viruses are often recognized as agents of disease and outbreaks occasioned by fecal-oral route through direct contact between people, and ingestion of contaminated food and water related to inadequate sanitary conditions and present in high concentrations in wastewater (Kittigul et al. 2005; Prado and Miagostovich 2014). They are associated with acute gastroenteritis and are a leading cause of infant mortality in underdeveloped countries. Mastadenovirus (AdV), rotavirus, norovirus, sapovirus, poliovirus, hepatitis A virus, and astrovirus are main infectious enteric viruses (Parashar et al. 2006; Bosch et al. 2008; Nakamura et al. 2016).

Human mastadenovirus (HAdV) and rotaviruses are responsible for several diseases that affect adults and children (Parashar et al. 2006; Vecchia et al. 2012; Prado and Miagostovich 2014; Radke and Cook 2018). Both can be isolated from the feces of asymptomatic patients for a prolonged period, and it can cause generalized infections in immunocompromised individuals (Vecchia et al. 2012; Radke and Cook 2018). Additionally, HAdV is associated with a wide variety of diseases such as respiratory, ocular, gastroenteric, and urinary pathologies (Kosulin et al. 2016; Radke and Cook 2018).

Anthropogenic disturbances are considered the main cause of biodiversity loss worldwide and can favor the spread of diseases, increasing the risk of spillover events (Ellwanger et al. 2022). Bats are even more likely speculated as unique in their potential to harbor zoonotic viruses due characteristics as flight, allowing movement and dispersal over long distances in some species (Luis et al. 2013). Moreover, many bat species are gregarious, some living in dense aggregations and cohabiting roosting sites with diverse assemblages of multiple bat species (Kuzmin et al. 2010; Luis et al. 2013). Additionally, their unique immune system limiting self-damaging inflammatory responses makes bats more likely to host zoonotic viruses in particular and/or transmit them to humans (Banerjee et al. 2020; Irving et al. 2021). Emerging and reemerging infectious diseases may be associated with viruses transmitted by bats being considered an important group for research programs of global interest (Li et al. 2010; Banerjee et al. 2020; Letko et al. 2020; Irving et al. 2021).

Some bat species established themselves in urbanized places and acquired synanthropic habits, which bring these species closer to humans and domestic animals. This behavior may pose a direct risk with virus spillover events among other animal species

including humans showing the capacity to act as reservoirs or viral infection sources (Luis et al. 2013; Banerjee et al. 2019; Letko et al. 2020). In addition, chiropterans show adaptive success in urban environments, with adjustments in foraging strategies, allowing them to thrive in search of different food sources and shelters, playing important roles as disseminators of pollen, seeds, and insect predators that can act like plagues (Egert-Berg et al. 2021). Thus, the present study aimed to verify the presence of adenovirus and rotavirus in fecal samples of chiropteran captured in Southern Brazil.

Methods

Samples

Rectal swabs samples were obtained in 60 bats of families Mollossidae (*Molossus molossus*, *M. rufus*), Phyllostomidae (*Sturnira lilium*, *Glossophaga soricina* and *Artibeus lituratus*), and Vespertilionidae (*Eptesicus brasiliensis* and *Histiotus velatus*) between September 2021 to July 2022 (Supplementary Table 1). The animals were captured with mist nets, collected, photographed and released at the same capture site. The rectal swabs of each animal were stored individually in tubes containing 1 mL of RNAlater™ Stabilization Solution (ThermoFisher Scientific™, Waltham, MA, United States), transported in an isothermal box and stored at -80º C until use.
The present study was approved by the Ethics Committee on Animal Use of Universidade Feevale (CEUA-FEEVALE) under protocol number #02.21.097 and authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) under protocol number #78499-1.

Nucleic acids extraction and Polymerase Chain Reaction (PCR)

RNA and DNA isolation were performed using MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific™) in the automatic KingFisher™ Duo Prime equipment (Thermo Fisher Scientific™), following the manufacturer's recommendations. The cDNA was synthesized using GoScript™ Reverse Transcriptase kit (Promega Corporation, Madison, WI, United States), following the manufacturer's recommendations.
A RT-PCR protocol in order to amplify a 160 bp fragment of viral protein 6 (VP6) of rotavirus

were performed using primers VP6 ROTA-F 5'-GATGTCCTGTACTCCTTGT-3' and VP6 ROTA-R 5'-GGTAGATTACCAATTCCTC-3' (Vecchia et al. 2012). For the detection of AdV, a nested PCR was used in order to amplify a fragment of 261 bp of the DNA polymerase, using the primers EF - POL-F 5'- CAGCCKCKGTTRTGYAGGGT-3' and ER - POL-R 5'-GCHACCATYAGCTCCAACTC- 3' in the first PCR round, and IF - POL-NF 5'-GGGCTCRTTRGTCCAGCA-3' and IR - POL-NR 5'-TAYGACATCTGYGGCATGTA-3' in the second reaction (Li et al. 2010).

A positive sample for HAdV-5 previously obtained in a human case and rotavirus vaccine (RotaTeq®, Merck Sharp & Dohme Pharmaceutica Ltda, São Paulo, SP, Brazil) were used as positive controls.

Purification, sequencing, and phylogenetic analysis

Partial amplification products of positive samples were purified using the PureLink™ PCR Purification Kit (Thermo Fisher Scientific™), following the manufacturer's recommendations. The sequencing of both strands was carried out at the ACTGene Laboratory (Alvorada, RS, Brazil) using the automatic sequencer ABI-PRISM 3100 Genetic Analyzer armed with 50 cm capillaries and POP6 polymer (Applied Biosystems, Waltham, MA, United States).

Genome edition was performed using the Geneious Prime 2022.1 bioinformatics suite program. The samples were compared with sequences available in the GenBank database using the nucleotide BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were aligned using the MAFFT program (Katoh and Standley 2013). Phylogenetic analysis was performed using the Maximum Likelihood (ML) inference model with the MEGA 11 program (Tamura et al. 2021). The replacement model was chosen for each hypothesis using the Find Best DNA/Protein model tool. The robustness of the hypothesis was tested in 1000 replicates.

Results

In the present study, 60 rectal swabs samples obtained in bats were analyzed by PCR where 13.3% (8/60) tested positive for AdV and all tested negative for rotavirus. AdV amplification products were submitted for DNA sequencing, as well as the positive control used to rule out cross-contamination.

The sequences obtained in the present study were deposited in GenBank database under accession number OQ689002 - OQ689009. The eight samples showed 76.7 to 100% of nucleotide identity between each other, with none of the samples showing 100% identity with the positive control used in the present study (LMM-HAdV-5), confirming the absence of cross-contamination (Figure 2). In nucleotide BLAST analyses, five samples showed 99.2 to 100% nucleotide identity with sequences classified as HAdV species E (HAdV-E), and three showed 98.6 to 99.6% with sequences classified as HAdV- C.

Two out the positive samples classified as HAdV-C (LMM23 and LMM26) and two as HAdV-E (LMM33 and LMM44) were obtained in *Glossophaga soricina* bats captured in the same colony in the municipality of Santo Antônio da Patrulha. These four sequences were not identical between each other. One sample classified as HAdV-C was obtained in a *G. soricina* from Eldorado do Sul (LMM7) and three other samples classified as HAdV-E were collected in *Eptesicus brasiliensis* from Campo Bom (LMM19), *Sturnira lilium* from Viamão (LMM15) and *Histiotus velatus* from Eldorado do Sul (LMM10).

The phylogenetic tree of the partial polymerase fragment was constructed using representative HAdV-A to G and AdV of other animal species sequences (Figure 2). The phylogenetic reconstruction presented bootstrap values ranging 93 to 100% in the nodes that delimit the species and genotypes grouping, reinforcing the robustness of the hypothesis. The samples detected in the present study were grouped into clades with sequences classified as HAdV-C and E.

Discussion

In the present study rectal swabs samples collect in molossid, phyllostomid, and vespertilionid bats were analyzed by PCR for AdV and rotavirus, where 13.33% tested positive for AdV and none tested positive for rotavirus. The AdV positive samples were submitted for DNA sequencing followed by phylogenetic analysis and classified as HAdV-C and HAdV-E (Figure 2). HAdV was detected in *Glossophaga soricina*, *Eptesicus brasiliensis*, *Histiotus velatus* and *Sturnira lilium* in the present study. HAdV-C has already been reported in *S. lilium* in Southern Brazil (Finoketti et al. 2019). However, reports of HAdV in bats are uncommon, with bat AdV being more frequent in different species of bats (Raut et al. 2012; Lima et al. 2013; Hackenbrack et al. 2017;

Iglesias-Caballero et al. 2018).

To the best of our knowledge, this is the first report of presence of HAdV in *Glossophaga soricina*, *Eptesicus brasiliensis* and *Histiotus velatus* bats. In addition, HAdV-C detection has been more reported in wildlife when compared to other HAdV genotypes, as observed in previous studies in bats (*Sturnira lilium*) (Finoketti et al. 2019) and in other wild animals such as cavies (*Cavia aperea aperea*) (Sita et al. 2022), pampas foxes (*Lycalopex gymnocercus*), crab-eating foxes (*Cerdocyon thous*) (Monteiro et al. 2015), white-eared opossum (*Didelphis albiventris*) (Menezes et al. 2020) and in American seals (*Arctocephalus australis*) (Chiappetta et al. 2017). In the present study, the presence of HAdV-E in bats was reported, which as reported above, is unusual and may suggest that other HAdV genotypes may also be harbored in wild animals as bats.

All bat species positive for HAdV presence reported in the present study are considered synanthropic, possibly using these disturbed habitats as passageways in search for food, water source or rest (Oliveira et al. 2020; Da Silva et al. 2021; Egert-Berg et al. 2021). A possible explanation for the presence of HAdVs in bat species may be the accidental ingestion of food, water, or inhalation of supposedly contaminated aerosols. In addition, the presence of AdV found in feces may be related to their constant frequency in the environment (Prado and Miagostovich 2014; Radke and Cook 2018).

All the bat samples analyzed in the present study tested negative for rotavirus. In Brazil, studies reported rotavirus presence in *Molossus molossus* and *Glossophaga soricina* bats (Asano et al. 2016). Furthermore, distinct rotavirus strains have been detected in fecal samples of fruit (*Rousettus aegyptiacus*) and insectivores bats (*Taphozous mauritianus*) in Kenya (Waruhiu et al. 2017) and South Korea (Kim et al. 2016). Rotaviruses are important causes of diarrhea and hospitalization among children worldwide (Parashar et al. 2006), and their investigation in eco-surveillance studies is important.

Conclusion

In the present study, HAdV were detected in *Glossophaga soricina*, *Eptesicus brasiliensis*, *Histiotus velatus* and *Sturnira lilium*. To the best of our knowledge, this is the first study to describe the presence of HAdV *G. soricina*, *E. brasiliensis* and *H. velatus* samples. Furthermore, the presence of HAdV-E in bats was reported, which is

unusual and may suggest that other HAdV genotypes in addition to HAdV-C may also be harbored by wild animals. The results obtained in the present study hypothesize possible exposure of synanthropic bats in contaminated environments, making them potential for the dissemination of enteric viruses to other ecosystems. The data generated in the present study reinforce the importance of eco-surveillance of viral agents related to diseases in humans and wild animals. In addition, it is essential to identify possible new hosts or reservoirs that increase the risk of spillover and dissemination of infectious pathogens, helping to prevent and control zoonotic diseases.

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Compliance with ethical standards

Ethical approval

The present study was approved by the Ethics Committee on Animal Use of Universidade Feevale (CEUA-FEEVALE) under protocol number #02.21.097 and authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) under protocol number #78499-1.

Human and animal rights

All applicable institutional and/or national guidelines for the care and use of animals were followed.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figure 1.

Geographical distribution of bat capture sites analyzed in the present study.

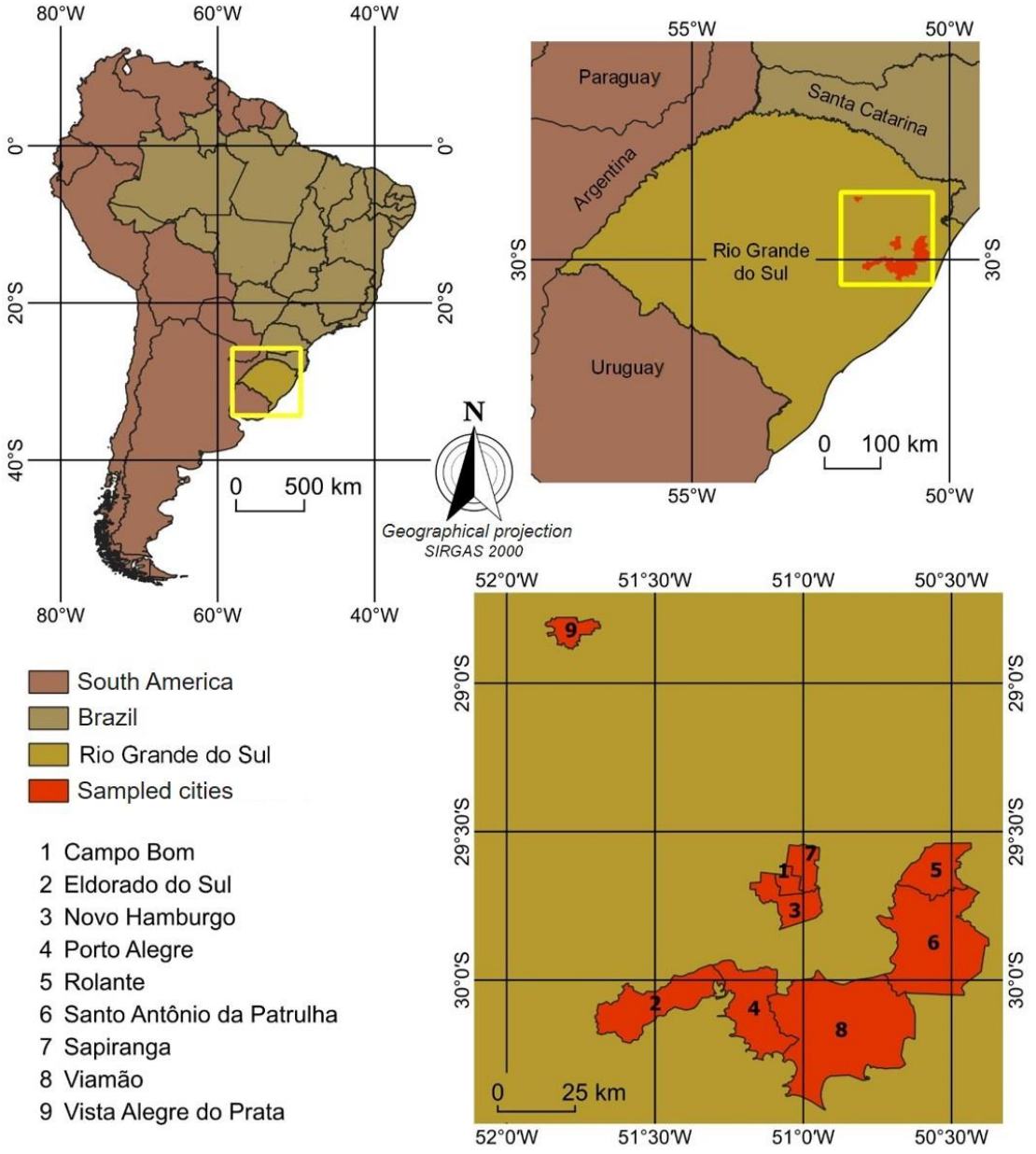
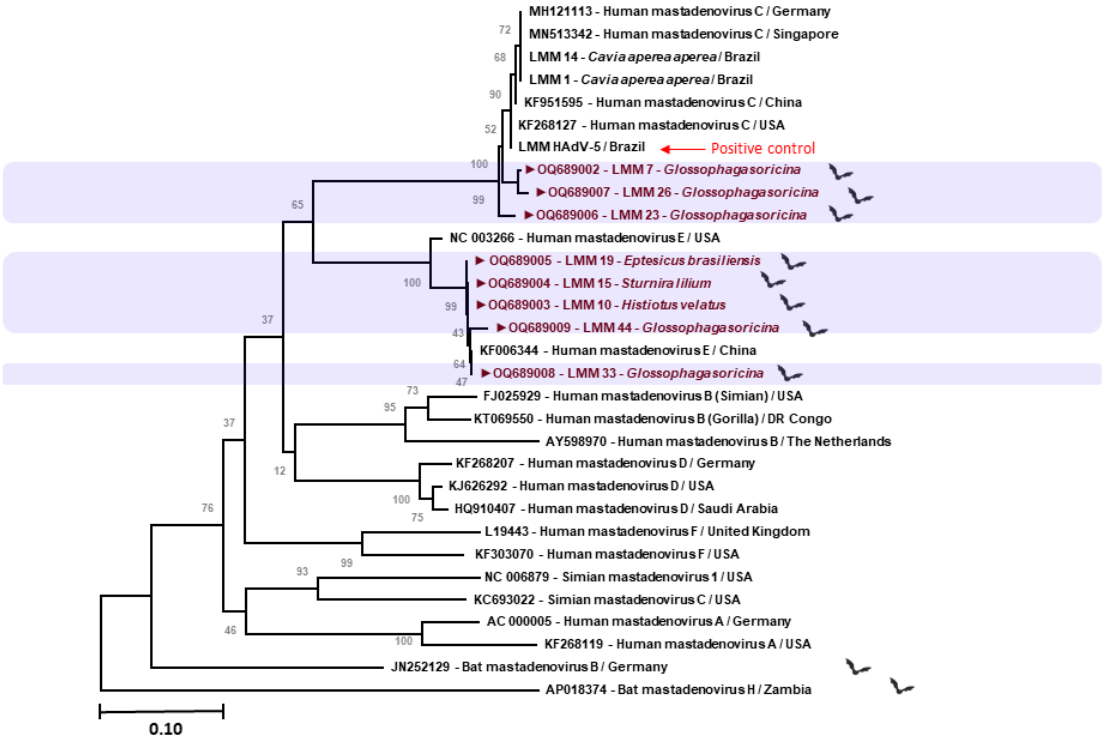


Figure 2.

Nucleotide phylogenetic tree constructed using adenovirus polymerase gene. The phylogenetic tree was constructed using MEGA 11 software using the maximum likelihood algorithm method based on the GTR+G+I model in 1000 replicates. The positive samples detected in the present study are highlighted in purple.



Supplementary Table 1

Data about the bat samples collected in the present study, including sampling sites, animal species, sex, bat diet and presence of adenovirus and rotavirus by nested PCR and RT-PCR, respectively.

Sample identification	Site	Species	Sex	Diet	*Adenoviridae*	*Reoviridae*
1	Sapiranga	*Molossus molossus*	M	In	-	-
2	Porto Alegre	*Artibeus lituratus*	F	Fr	-	-
3	Eldorado do Sul	*Glossophaga soricina*	F	Ne	-	-
4	Eldorado do Sul	*Glossophaga soricina*	F	Ne	-	-
5	Eldorado do Sul	*Glossophaga soricina*	F	Ne	-	-
6	Eldorado do Sul	*Glossophaga soricina*	M	Ne	-	-
7	Eldorado do Sul	*Glossophaga soricina*	M	Ne	+	-
8	Novo Hamburgo	*Molossus molossus*	F	In	-	-
9	Eldorado do Sul	*Histiotus velatus*	M	In	-	-
10	Eldorado do Sul	*Histiotus velatus*	F	In	+	-
11	Eldorado do Sul	*Histiotus velatus*	M	In	-	-
12	Eldorado do Sul	*Histiotus velatus*	F	In	-	-
13	Vista Alegre do Prata	*Sturnira lilium*	F	Fr	-	-
14	Viamão	*Artibeus lituratus*	M	Fr	-	-
15	Viamão	*Sturnira lilium*	F	Fr	+	-
16	Viamão	*Molossus molossus*	M	In	-	-
17	Viamão	*Molossus rufus*	M	In	-	-
18	Campo Bom	*Molossus molossus*	F	In	-	-
19	Campo Bom	*Eptesicus brasiliensis*	M	In	+	-
20	Campo Bom	*Eptesicus brasiliensis*	F	In	-	-
21	Rolante	*Sturnira lilium*	M	Fr	-	-
22	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
23	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	+	-
24	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
25	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
26	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	+	-
27	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
28	Santo Antônio da Patrulha	*Glossophaga soricina*	M	Ne	-	-
29	Rolante	*Glossophaga soricina*	M	Ne	-	-
30	Rolante	*Glossophaga soricina*	M	Ne	-	-
31	Santo Antônio da Patrulha	*Glossophaga soricina*	M	Ne	-	-
32	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
33	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	+	-
34	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
35	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
36	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
37	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
38	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
39	Santo Antônio da Patrulha	*Glossophaga soricina*	M	Ne	-	-
40	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
41	Santo Antônio da Patrulha	*Glossophaga soricina*	M	Ne	-	-

42	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
43	Santo Antônio da Patrulha	*Glossophaga soricina*	M	Ne	-	-
44	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	+	-
45	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
46	Santo Antônio da Patrulha	*Glossophaga soricina*	F	Ne	-	-
47	Novo Hamburgo	*Molossus molossus*	F	In	-	-
48	Novo Hamburgo	*Molossus molossus*	F	In	-	-
49	Novo Hamburgo	*Molossus molossus*	F	In	-	-
50	Novo Hamburgo	*Molossus molossus*	M	In	-	-
51	Novo Hamburgo	*Molossus molossus*	F	In	-	-
52	Novo Hamburgo	*Molossus molossus*	F	In	-	-
53	Novo Hamburgo	*Molossus molossus*	M	In	-	-
54	Novo Hamburgo	*Molossus molossus*	F	In	-	-
55	Novo Hamburgo	*Molossus molossus*	F	In	-	-
56	Novo Hamburgo	*Molossus molossus*	F	In	-	-
57	Novo Hamburgo	*Molossus molossus*	F	In	-	-
58	Novo Hamburgo	*Molossus molossus*	F	In	-	-
59	Novo Hamburgo	*Molossus molossus*	F	In	-	-
60	Campo Bom	*Artibeus lituratus*	M	Fr	-	-

M: male
F: female
In: insectivorous
Fr: Frugivorous
Ne: Nectarivorous
+: positive sample
- : negative sample

5. DISCUSSÃO GERAL E PERSPECTIVAS

Mudanças ambientais nos ecossistemas silvestres, principalmente pela antropização, têm diminuído habitat de várias espécies silvestres, o que faz com que estes animais cada vez mais ocupem as áreas urbanas e se aproximem das residências humanas, sendo capazes de ocasionar novas zoonoses. Contudo, também é fundamental a identificação correta dos hospedeiros reservatórios para possíveis programas de monitoramento.

Os roedores e quirópteros representam uma fonte importante no surgimento ressurgimento de patógenos zoonóticos com ligações complexas entre saúde de humanos e outros animais, embora tenha uma compreensão limitada sobre vírus que carregam e o que impulsionam o transbordamento para outras espécies. No presente estudo, foi possível a identificação de agentes virais relacionados a patógenos humanos em roedores e quirópteros, o que auxilia na ecovigilância no País. Dessa forma, a detecção de HAdV-C em preás, e de HAdV-C e E em morcegos, auxiliam no conhecimento de animais silvestres que podem albergar e talvez até transmitir patógenos humanos. Os resultados obtidos nos dois capítulos da presente dissertação demostram uma possível exposição desses animais em ambientes contaminados, tornando-os potencial para disseminação de vírus entéricos para outros ecossistemas.

O escopo do trabalho segue em andamento, com mais vírus de diferentes famílias sendo investigados, além da submissão das amostras estudas para sequenciamento de alto desempenho. As atividades acima citadas irão compor futura tese de doutorado do acadêmico.

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