SÃO PAULO STATE UNIVERSITY (UNESP)
SCHOOL OF VETERINARY MEDICINE AND ANIMAL SCIENCE, BOTUCATU

MOLECULAR SURVEY AND CHARACTERIZATION OF SELECTED VIRUSES IN WILD BRAZILIAN FELIDS

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Botucatu – SP
2024

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Thesis submitted to the Postgraduate Program in Wild Animals of the School of Veterinary Medicine and Animal Science of the São Paulo State University to obtain the Master's degree in Sciences.

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Botucatu – SP
2024

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSANGELA APARECIDA LOBO-CRB 8/7500

Colpani, Michelle.
Molecular survey and characterization of selected viruses in wild brazilian felids / Michelle Colpani. - Botucatu, 2024

Dissertação (mestrado) - Universidade Estadual Paulista (UNESP), Faculdade de Medicina Veterinária e Zootecnia, Botucatu
Orientador: José Luiz Catão-Dias
Capes: 50500007

1. Cardiovirus. 2. Felídeos. 3. Virus de RNA. 4. Rotavírus. 5. Infecções por Astroviridae.

Palavras-chave: Astrovirus; Cardiovirus; *Felidae*; RNA virus; Rotavirus.

SCIENTIFIC IMPACT OF THIS PROJECT

The outcomes of this survey will add to the limited knowledge of viruses circulating in wild felids from Brazil. The selected RNA viruses (i.e., *Astrovirus*, *Cardiovirus* and *Rotavirus*) can infect a wide range of host species, including human, wild, and domestic animals; thus, investigating their occurrence can help prevent potential outbreaks. Most reports on these viruses have pigs or rats as hosts, limiting our knowledge of their interaction with other hosts such as felids. The few reports on these selected viruses and felids as hosts are on African and Asian felid species, with no available information on other species. Molecular screening for these pathogens in neotropical felids has not been done yet, which highlights the importance of this project. Monitoring infectious diseases in wild mammals can also provide valuable results within the One Health scope. Due to the phylogenetic and evolutionary closeness between wild and domestic carnivores, information on the possible occurrence of infectious agents affecting these species is crucial for mapping potential host exchange occurrences. Since domestic dogs and cats accompany humans globally, it is important to monitor pathogens that may affect these animals and their non-domestic counterparts. Nevertheless, the selected viruses have a zoonotic potential, so its surveillance is a matter of public health. Therefore, identifying the occurrence or absence of these virus in Brazilian wild felids is very important for the species conservation and to prevent potential outbreaks originating from these viruses whether in humans or animals, as previously reported in other countries.

Author: **Michelle Colpani**

Title: **MOLECULAR SURVEY AND CHARACTERIZATION OF SELECTED VIRUSES IN WILD BRAZILIAN FELIDS**

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RESUMO

O presente estudo objetivou pesquisar a presença de astrovírus, cardiovírus e rotavírus em felídeos não domésticos, mantidos sob cuidados humanos ou em vida livre, em múltiplos biomas brasileiros, incluindo Amazônia, Cerrado, Caatinga, Mata Atlântica e zonas de transição. Os vírus selecionados são amplamente distribuídos em humanos e mamíferos domésticos, com infecções sintomáticas ou não. São agentes altamente resistentes às condições ambientais, com potencial zoonótico, e que já foram previamente associados a mortalidades e surtos. Para pesquisa desses vírus, instituições foram contatadas para fornecimento de amostras biológicas (e.g., sangue total, *swab* oral, *swab* retal, tecidos), as quais foram congeladas a -80ºC até a extração do RNA. A detecção de astrovírus foi realizada via transcriptase reversa seguida de reação em cadeia polimerase (RT-PCR), com posterior sequenciamento genético. Cardiovírus e rotavírus foram ambos testados em RT-PCR em tempo real, também seguidos de sequenciamento genético. De todos os 50 animais avaliados, apenas um gato do mato pequeno do sul (*L. guttulus*) mantido sob cuidados humanos foi positivo para astrovírus, enquanto uma suçuarana (*P. concolor*) de vida livre atropelada foi positiva para *Rotavirus A*. Nenhum animal foi positivo para cardiovírus. Análises morfológicas de tecidos fixados em formalina foram realizadas para avaliar possíveis lesões decorrentes da infecção viral por rotavírus, com resultados inconclusivos devido à autólise do material. Considerando o caráter zoonótico dos vírus selecionados para estudo, os resultados deste trabalho, além de contribuírem para saúde pública no escopo de Saúde Única, auxiliarão na melhor compreensão das infecções virais que podem acometer felídeos selvagens.

Palavras-chave: Astrovírus; Cardiovirus; Felidae; RNA vírus; Rotavirus

ABSTRACT

The aim of this study was to investigate the presence of astrovirus, cardiovirus and rotavirus in non-domestic felids under human care or in the wild in multiple Brazilian biomes, including Amazon Forest, Atlantic Forest, Caatinga, Cerrado and its transition zones. The selected viruses are widely distributed in humans and domestic mammals, whether with clinical manifestation or not. They are highly resistant to environmental conditions, may have zoonotic potential and have already been associated with fatal outbreaks. To survey these viruses, institutions were contacted to supply biological samples (e.g., blood, oral swabs, rectal swabs, and tissues) which were frozen at -80ºC until RNA extraction. Astrovirus detection was performed via reverse transcriptase followed by polymerase chain reaction (RT-PCR), with subsequent genetic sequencing. Cardiovirus and rotavirus were both tested using real time RT-PCR, also followed by genetic sequencing. Of all 50 analyzed felids, one captive southern tiger cat (*L. guttulus*) tested positive for astrovirus while one roadkill cougar (*P. concolor*) tested positive for *Rotavirus A*. All felids were negative for cardiovirus. Morphological analyses of available formalin-fixed tissues of the rotavirus PCR-positive case were conducted to search for possible lesions caused by the virus, but results were inconclusive due to the autolysis. Considering the zoonotic nature of these viruses, the results of this work add to the current knowledge on viruses circulating in non-domestic felids in Brazil, which can contribute to public health within the scope of One Health and help to better elucidate viral infections that can affect wild felids.

Key words: Astrovirus; Cardiovirus; Felidae; RNA virus; Rotavirus

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INTRODUCTION

1 INTRODUCTION

The last recent decades have been marked by the COVID-19 pandemic and other important epidemics (e.g., Ebola, Zika virus, avian flu, swine flu), all caused by RNA viruses (Qureshi et al., 2023). Viruses are the main infectious agents linked to human disease outbreaks, especially foodborne, so they are considered a high-priority subject of study (Olaimat et al., 2024). There is evidence that around 60% of human infections are zoonotic, and 70% of these have a wildlife origin (Kuczera et al., 2024). The emergence of novel infectious diseases in both human and wild animals has been related to anthropogenic environmental changes, mainly linked to habitat fragmentation, environment degradation and introduction of new invasive exotic species (Smith, Acevedo-Whitehouse & Pedersen, 2008). Consequently, infectious diseases are significant threats to wildlife, as they can increase some species' risk of extinction (Smith, Acevedo-Whitehouse & Pedersen, 2008) and should be constantly monitored.

Among wild animals, carnivores (Order Carnivora) have a higher risk of extinction mediated by infectious diseases due to their close phylogenetic relatedness with their domestic counterparts [e.g., dogs (*Canis lupus familiaris*) and cats (*Felis catus*)], which accompany humans globally (Smith, Acevedo-Whitehouse & Pedersen, 2008). There is a particular concern about members of the Felidae family, as over 70% of their species are facing population decline related to direct or indirect human interaction. Furthermore, they present a generally low reproductive success and occur in naturally low densities (Johansson et al., 2020; IUCN, 2024). Thus, surveying for infectious agents that may affect wild felids is of great importance for species conservation.

Habitat fragmentation reduces prey availability for wild felids and their original large home ranges, driving them and other wild animals to areas with abundant resources not susceptible to seasonal fluctuations (e.g., urban and peri-urban areas) (Bradley & Altizer, 2006; Foley et al., 2013). This way, anthropogenic environmental changes allow humans, domestic, and wild animals to share territories, increasing the chances of intra and interspecific transmission of different pathogens among them (Pacini et al., 2023). Environmental degradation caused by human practices can also interfere with wild felids' social organization, as it may increase rates of infanticide and endogamy

(Johansson et al., 2020). Animals resulting from inbreeding are more prone to express recessive mutations and, therefore, have a higher likelihood of developing a more fragile immune system (Smith, Acevedo-Whitehouse & Pedersen, 2008). When challenged by pathogens, these animals have a lower chance of recognizing them and act as pathways for "new" infectious pathogens to enter healthy populations (Smith, Acevedo-Whitehouse & Pedersen, 2008).

The introduction of new invasive exotic species can also impact native wildlife health (Smith, Acevedo-Whitehouse & Pedersen, 2008). In Brazil, there are at least nine wild felids species, such as the ocelot (*Leopardus pardalis*), the margay cat (*Leopardus wiedii*), the jaguarundi (*Herpailurus yagouaroundi*), the oncilla cat (*Leopardus tigrinus*), the Geoffroy's cat (*Leopardus geoffroyi*), the southern tiger cat (*Leopardus guttulus*), the pampas cat (*Leopardus colocolo*), and two species considered large felids, the jaguar (*Panthera onca*) and the cougar (*Puma concolor*) (IUCN, 2024). As predators, wild felids maintain their biome's ecosystem balance by regulating prey density (Labarge et al., 2021). The major diet components of Brazilian wild felids are wild and domestic rodents and suids, including exotic species (i.e. wild boars [*Sus scrofa*]) (Seward et al., 2004; Cavalcanti et al., 2010; Adania, Silva & Felippe, 2014). Suids and rodents are reservoirs of many viral pathogens, including astroviruses, cardioviruses and rotaviruses (Billinis, 2009; Zhuang et al., 2023; Gangopadhayya et al., 2024). All these organisms are RNA viruses with high recombination rates that can cause diseases in a wide host range, with or without clinical signs, including humans (Werid et al., 2022a), which justifies the need of further investigation on a One Health approach.

There are few reports regarding the occurrence of any of the selected viruses proposed in the current research in wild felids, and they are mainly from exotic species, with no reports on neotropical felids (Atkins et al., 2009; Liu et al., 2013; Johansson et al., 2020). Considering the relevance of wild felids conservation for ecosystem maintenance, their impact within the One Health context (Labarge et al., 2021), and recent global events, the present study aims to survey selected RNA viruses (i.e., astrovirus, cardiovirus, and rotavirus) in wild Brazilian felids. To the author's knowledge, this is the first molecular screening of these viruses in wild felids species from America, including at least one vulnerable species, the southern tiger cat (IUCN, 2024), and the work aims to contribute to the felid's conservation within a One Health perspective.

OBJECTIVES

2 OBJECTIVES

The main goal of this project is to use biomolecular techniques to search for selected RNA viruses (i.e., astrovirus, cardiovirus and rotavirus) in wild Brazilian felids.

More specifically, this research aims to:

a – Investigate and characterize by reverse transcriptase polymerase chain reaction (RT-PCR) the occurrence of astrovirus in biological samples from free-ranging and captive wild Brazilian felids;

b – Investigate by real time RT-PCR (RT-qPCR) the occurrence of cardiovirus in biological samples from free-ranging and captive wild Brazilian felids;

c – Investigate by RT-qPCR the occurrence of rotavirus in biological samples from free-ranging and captive wild Brazilian felids;

d – Characterize eventual histopathological lesions in positive cases;

e – Dispose data for future epidemiological analysis on felid's conservation by reporting the occurrence or absence of the selected viruses in wild felids considering multiple epidemiological variables, including most compromised *taxa*, animal's place of origin, gender, age, and biological and clinical history, when available.

LITERATURE REVIEW

3 LITERATURE REVIEW

3.1 *Astrovirus*

Astroviruses (AstV) are small (28 to 40 nm), non-enveloped, rounded positive-sense single-stranded RNA viruses (Santos & Cardoso, 2005). They were named after their unique morphology, resembling a five-to-six-pointed star when visualized under electron microscopy (Greene, 2015). AstV belong to the *Astroviridae* family, which currently is divided in two genera: *Avastrovirus*, mainly detected in avian hosts, and *Mamastrovirus* in mammalians (Li et al., 2021). However, *Avastrovirus* has already been found in mammals, and there are also reports of *Mamastrovirus* in avian hosts (Huang et al., 2024). Additionally, different AstV may coinfect the same host cell, increasing the chances of recombination between different strains (Guix, Bosch & Pintó, 2013). As a result, AstV exhibit high mutation rates and genetic diversity, which facilitates virus transmission to new host species (Huang et al., 2024; Kuczera et al., 2024). Furthermore, recombination events are extremely common in RNA viruses and have notably increased, including in AstV (Wang et al., 2020). Based on molecular data a novel classification criterion has been suggested by the International Committee on Taxonomy of Viruses (ICTV) not associated with host taxonomy. Currently, strain classification is based on phylogenetic analysis of the ORF2 gene, and ICTV officially recognizes 19 species within *Mamastrovirus* genera (MAstV-1 to MAstV-19) (Bosch et al., 2009). For instance, Feline astrovirus (FeAstV) was identified shortly after human astrovirus (HAstV) and is classified as MAstV-2 by the ICTV (Dong et al., 2021).

The AstV genome has approximately 6,400 and 7,700 base pairs (bp) in length, subdivided into open reading frames ORF1a, ORF1b and ORF2 (Santos & Cardoso, 2005). Regions ORF1a and ORF1b are the most preserved and encode the non-structural protein RNA-dependent RNA polymerase (RdRp), which is essential for viral replication (Guix, Bosch & Pintó, 2013). ORF2 encodes the precursors of capsid proteins and thus determines the virus's immunogenicity (Yi et al., 2018; Wang et al., 2020). The viral capsid is usually the region that endures most host immune system pressure and, therefore, is generally more prone to mutations than RdRp (Atkins et al., 2009). Overall,

AstV are thought to have high biological plasticity because they can infect a wide range of hosts species, complicating its diagnostics (Van Brussel et al., 2020; Caetano et al., 2021).

Round, small viruses like AstV can only be visualized with electron microscopy when in high concentrations of viral particles (Greene, 2015). Since 1990 molecular techniques have been prioritized as diagnostic tools because of their superior sensibility and specificity (Santos & Cardoso, 2005; Greene, 2015). Therefore, RT-PCR followed by genetic sequencing is now considered the gold standard method for AstV detection and characterization (Santos & Cardoso, 2005; Lawler et al., 2018). Different RT-PCR protocols have been used worldwide, including nested reactions, heminested, and RT-qPCR (Atkins et al., 2009; Lawler et al., 2018; Wang et al., 2021). Furthermore, molecular techniques allow for viral genomic phylogenetic analysis, which can be useful for distinguishing AstV of animal origin from those of human origin (Santos & Cardoso, 2005; Lawler et al., 2018). For instance, recent phylogenetic analysis revealed that FeAstV is the animal AstV most closely related to HAstV (Li et al., 2021), raising concerns about its potential zoonotic transmission.

3.1.1 Enteric astroviruses

Astroviruses were first isolated in feces and lately from vomit samples obtained from domestic cats, suggesting that replication may occur in both upper and lower gastrointestinal tracts (Li et al., 2021). The incubation period for enteric AstV varies from three to five days (Olaimat et al., 2024). Clinical manifestations typically appear between two to three days, primarily including gastrointestinal disturbances, such as mild to severe watery diarrhea (Nguyen, Piewbang & Tchangamsuwan, 2023). Additionally, weight loss, anorexia, severe weakness, inappetence, and vomiting have also been reported in domestic cats (Wang et al., 2021; Olaimat et al., 2024). However, in most cases, infection is asymptomatic, and when clinical manifestation does occur, it is usually mild and self-limiting (Santos & Cardoso, 2005). The most severe cases in domestic cats have been associated with coinfection with other viruses (e.g., parvovirus), highlighting the need for further research to determine whether AstV infections are primary or opportunistic (Yi et al., 2018; Van Brussel et al., 2020). Diarrhea can progress and potentially become fatal, particularly in kittens or young and immunosuppressed animals (Lawler et al., 2018; Zhang et al., 2019).

FeAstV infections have been reported worldwide, with occurrence raging between 23 and 25% among domestic cats (Yi et al., 2018; Zhang et al., 2019). Moreover, diarrhea has been statistically associated with FeAstV infection (Dong et al., 2021). On another hand, asymptomatic cats can also excrete viral particles for up to 20 days, underscoring the need for periodic surveillance regardless of clinical manifestations (Li et al., 2021).

Diarrhea is also a leading cause of mortality in children and immunosuppressed young people (Santos & Cardoso, 2005). Enteric viral agents are highly relevant, with HAstV from species MAstV-1 being considered the second most frequent enteric viruses in children (Olivares et al., 2020). Affected individuals may display no symptoms at all or experience watery diarrhea lasting up to three days, along with nausea, vomiting, and abdominalgia (Pérot, Lecuit & Eloit, 2017). Symptoms caused by AstV are usually self-limiting and less severe than those caused by rotaviruses (Pérot, Lecuit & Eloit, 2017; Olivares et al., 2020). Some reports have identified AstV strains in humans with greater similarity to animal AstV than to HAstV, suggesting a potential risk of new emerging zoonotic infections caused by AstV of animal origin (Nguyen, Piewbang & Techangamsuwan, 2023; Kuczera et al., 2024).

3.1.2 Neurotropic astroviruses

Besides classic gastrointestinal astroviruses, there is also the possibility of systemic clinical manifestation (Kuczera et al., 2024). Atypical AstV strains have been identified in the brains of children who were later diagnosed with encephalitis, suggesting an emerging infection that could be possibly fatal in children and/or immunosuppressed patients (Lum et al., 2016). Since neuroinvasive AstV are highly divergent from their classic enteric forms, phylogenetic analysis suggests a possible mutation event that may have resulted in different viral tropism (Lum et al., 2016). However, coinfections of both enteric and neurotropic strains in the same host are not unusual (Boros et al., 2017). Neurotropic AstV strains are classified in humans as HAstV-MLB1-3 and belong to the MAstV-6 species (Olivares et al., 2020; Caetano et al., 2021), also named as non-classic HAstV. Both classic and non-classic AstV have already been identified in fecal samples from children in the Brazilian Amazon since 1990 (Olivares et al., 2020). In Goiania (GO), there are also reports of AstV occurrence, particularly between September and March, when there are higher rainfall indices (Santos & Cardoso, 2005).

Fatal encephalitis is a common finding in AstV neurotropic infections in other mammal species, especially young and immunosuppressed animals (Lum et al., 2016; Boros et al., 2017). In piglets, an AstV extremely similar to classic porcine astrovirus (PoAstV) was related to hind limb paresis, muscular weakness, and loss of consciousness (Boros et al., 2017). Furthermore, since FeAstV and PoAstV share a common ancestor (Wang et al., 2020), there is growing questioning about the potential for neuroinvasive AstV in other animals (Wildi & Seuberlich, 2021), such as wild felids.

3.1.3 Astrovirus transmission, control and prevention

Astroviruses transmission occurs mainly by fecal-oral route (Zhang et al., 2019). Ingestion of contaminated water or food by humans or animal feces is the most common way of AstV infection in humans (Olaimat et al., 2024). For instance, AstV particles have been detected at significant rates in sewage water worldwide (Hoque et al., 2023; Kumthip et al., 2023; Rao et al., 2023; Zamora-Figueroa et al., 2024). Besides the fecal-oral route, direct transmission through intraspecific (e.g., mating, socialization, fighting) and interspecific contact between wild or domestic preys, carnivores and scavengers are other main forms of AstV infection in animals (Johansson et al., 2020). AstV strains phylogenetically close to those found in foxes, bats and birds have been identified in the feces of domestic cats, suggesting possible ingestion of infected prey (Lawler et al., 2018). Ingestion of contaminated water sources, carcasses, and territorial marking are some examples of indirect AstV transmission pathways, particularly in areas close to human habitations (Johansson et al., 2020). Tourism and high traffic of people in tourist areas, such as the Amazon Forest, may introduce new AstV strains into the Brazilian scenario or other biomes (Olivares et al., 2020). Furthermore, animal AstV strains have been identified in feces of *Aedes aegypti* adult females in India, although the significance of this finding needs further investigation (Gangopadhayya et al., 2024).

Treatment for AstV infection in humans or animals focuses only on managing clinical manifestations (Wang et al., 2021). There is currently no vaccine available against AstV, thus, biosecurity measures are the most effective form of prophylaxis (Wildi & Seuberlich, 2021).

3.1.4 Astrovirus in wild felids

The first report of a possible new FeAstV variant strain in non-domestic felids was in a population of cheetahs (*Acinonyx jubatus*) kept under human care in Florida (EUA) (Atkins et al., 2009). Seven adult animals presented lethargy, anorexia, watery diarrhea, and regurgitation; five of them shared the same enclosure but two affected animals had no direct contact with the others (Atkins et al., 2009). A pan-AstV hemi nested RT-PCR protocol was established and used on fecal samples with universal primers targeting gene ORF1b and then ORF2 (Atkins et al., 2009). Phylogenetic analysis revealed high nucleotide identity similarity with HAstV-5 and FeAstV, respectively.

AstV was later identified in oral swabs and fecal swabs from two adult tigers (*Panthera tigris*) with severe drooling resistant to antibiotic therapy, both sharing the same enclosure at a Chinese zoo (Zhang et al., 2019). Phylogenetic analysis based on RdRp fragments suggested TigAstV as a novel AstV strain that possibly shares a common origin with the previously known FeAstV (Zhang et al., 2019). Furthermore, when comparing the nucleotide's identity of cheetahs, tigers, and domestic cats, authors also suggest possible interspecific transmission between animals within the family Felidae (Atkins et al., 2009; Zhang et al., 2019), but further investigations on AstV genomic sequences in other felids species are needed to clarify this.

Nonetheless, viral particles with weak homology to AstV were identified by molecular techniques in rectal swabs from two of the eight free-ranging adult snow leopards (*Panthera uncia*) tested in Mongolia (Johansson et al., 2020).

Although the impact of AstV on wild animal health is not yet fully established, it is believed that it still poses a threat, particularly to individuals under the cumulative effects of stress factors and consequent immunosuppression (Pacini et al., 2023), as wild felids. Therefore, molecular screening surveys and pathological investigations are needed to further understand AstV infection in wild felids, especially considering that FeAstV is phylogenetically close to the possible neuroinvasive PoAstV and to HAstV (Soma et al., 2020; Wang et al., 2020). In addition, to its date, there are no reports of AstV screening on Brazilian felids, indicating a need for further investigation since current available literature focuses on Old World felid species.

3.2 *Cardiovirus*

Cardiovirus is also known as encephalomyocarditis virus (EMCV), as it can cause a homonymous disease (Lu et al., 2021). They belong to the genus *Cardiovirus* (Family *Picornaviridae*), currently with seven species named *Cardiovirus A* to *F* (Foglia et al., 2023). These are small (30 nm), non-enveloped, rounded positive-sense single-stranded RNA viruses, highly resistant to hostile environment (Carocci & Bakkali-Kassimi, 2012). Its genome is approximately 7,800 pb in length subdivided in two ORFs that encode a total of 13 structural and non-structural proteins (Carocci & Bakkali-Kassimi, 2012). Genes 1A-D encode structural capsid proteins, known as VP4, VP2, VP3, and VP1 respectively, with VP1 being responsible for strain virulence (Carocci & Bakkali-Kassimi, 2012; Vyshemirskii et al., 2017). Nonstructural proteins are encoded by genes 2A (important for viral translation), 2B (high genetic variation), 2C, and 3A-D, all of which are involved in viral replication (Carocci & Bakkali-Kassimi, 2012; Romey et al., 2021). Gene 3D experiences less selective pressure from the host immune system, making it a highly conserved gene and a good target for molecular analysis (Koenen et al., 1999). Gene 1D (VP1), on the other hand, is responsible for virus adherence to the host cell receptor and is typically used to further classify EMCV serotypes into 1 to 4 (Vyshemirskii et al., 2017). However, some authors believe mutations in gene 1D (VP1) are silent because EMCV strains found in different animal species in Europe, Asia and South America are highly similar (Oberste et al., 2009; Cardetti et al., 2016; Medkour et al., 2021). Thus, EMCV has emerged worldwide as a new sporadic pathogen with high antigenic and molecular stability, capable of infecting a wide range of host species (Romey et al., 2021; Foglia et al., 2023).

The pathogenesis of EMCV is still under investigation, but experimental reports suggest that this virus has a tropism for receptors located in vascular endothelial cells (Lipton, Kumer & Hertzler, 2007). Once EMCV attaches to the host cell, viral RNA acts as mRNA during translation and as a template during genomic replication, which occurs within host cell's cytoplasm (Knowles et al., 2009). New viral particles are then released within the host cell, leading to an exacerbated inflammatory response mediated by the host's immune system (Carocci & Bakkali-Kassimi, 2012). Excessive inflammatory cytosines can increase EMCV virulence and negatively affect cardiac function, leading to acute non-suppurative myocarditis that may result in death (McLelland et al., 2005). Indeed, EMCV infections are usually fatal, and sudden death is

typically considered its main clinical manifestation, as reported in non-humans primates and animals from the families Camelidae, Elephantidae, Felidae, Hippopotamidae, Macropodidade, Tapiridae and Suidae (Koenen et al., 1999; Oberste et al., 2009; Canelli et al., 2010; Liu et al., 2013; Cardetti et al., 2016; Flacke et al., 2016; Vercammen et al., 2017; Vyshemirskii et al., 2017; O'Connor et al., 2020; Medkour et al., 2021; Romey et al., 2021). Other clinical manifestations in different animal species include encephalitis, lethargy, weakness, cramps, shakiness, dyspnea, and motor incoordination (Canelli et al., 2010; Cardetti et al., 2016; O'Connor et al., 2020; Foglia et al., 2023; Gris et al., 2023). However, some animals can be infected by EMCV and display no evident clinical manifestation (Carocci & Bakkali-Kassimi, 2012; Fernandez-Cassi et al., 2020; Ao, Xu & Duan, 2022; Werid et al., 2022b; Grist et al., 2023).

Most EMCV cases are reported in animals kept under human care in locations with a history of rodent infestation (O'Connor et al., 2020; Romey et al., 2021). Rodents are generally considered the natural definitive and intermediary hosts in EMCV ecology, as well as their reservoirs, because they can excrete viral particles for up to 29 days (Lipton, Kumer & Hertzler, 2007; Truong et al., 2013; Kishimoto et al., 2021; Gris et al., 2023). Although the main transmission pathway of EMCV is through the fecal-oral route, viral particles have also been identified in extra-intestinal tissues of rodents, suggesting it can be transmitted not only through ingestion of contaminated feces but also the whole prey (Reddacliff et al., 1997; Boros et al., 2019). Considering that wild and domestic rodents are the primary prey of wild Brazilian felids, they pose a risk to felids' health by acting as reservoirs of several pathogens (Seward et al., 2004; Cavalcanti et al., 2010; Adania, Silva & Felippe, 2014). Additionally, vertical transmission of EMCV has been reported in pigs, but the most common method of infection is horizontal through ingestion of water or food contaminated by infected rodent feces (Vyshemirskii et al., 2017; Gris et al., 2023). High quantities of *Cardiovirus* viral particles have been detected in sewage water worldwide (Stockdale et al., 2023; Li et al., 2024).

EMCV is a potential zoonotic virus that when infecting humans may result or not in clinical manifestations, such as encephalitis and aseptic meningitis (Lipton, Kumer & Hertzler, 2007; Feng et al., 2015; Foglia et al., 2023). Some authors suggest that humans can become infected if open wounds come into contact with infected animals, especially domestic cats, as they are more likely to interact with rodents (Oberste et al., 2009; Li et al., 2024).

In 1960, an EMCV epizootic occurred in the Brazilian Amazon Forest, confirmed after antibodies against EMCV were detected in wild rodents, horses, cows, birds and mosquitoes (Causey, Shope & Laemmert, 1962). Later, in 1980, there was an EMCV outbreak on a pig farm in Southern Brazil, with a reemergence in 2023 on another farm in the East Center of Brazil (Gris et al., 2023). This last report characterizes the first molecular description of EMCV in Brazil (Gris et al., 2023).

Managing environmental contamination with EMCV is challenging, thus, prophylactic measures are the best way to prevent potential outbreaks, including control of rodent population, periodical cleaning of animal enclosures and serologic testing for anti-EMCV antibodies before transferring animals to new institutions (Lipton, Kumer & Hertzler, 2007). For instance, some authors suggest that EMCV introduction into certain zoos may have occurred through wildlife exchange among institutions (McLelland et al., 2005; Lipton, Kumar & Hertzler, 2007; Flacke et al., 2016). Vaccines are still under development, however, serologic surveys in healthy animals suggest that a prior exposure to EMCV does not protect them from subsequent infections (Reddacliff et al., 1997; Backues et al., 1999; McLelland et al., 2005).

Therefore, considering the mortality rate associated with EMCV and its capacity to cause outbreak in various host species, including humans, surveillance of EMCV in wild Brazilian felids is a matter of public health within the One Health scope (Yuan et al., 2014; Feng et al., 2015).

3.2.1 *Cardiovirus* in wild felids

Within the Felidae family, antibodies against EMCV were first reported in a healthy lion (*Panthera leo*) kept in a zoo where a recent EMCV outbreak had caused the sudden death of several other animals from different species [e.g., non-primate species, pygmy hippopotamus (*Choeropsis liberiensis*) and goodfellows tree kangaroos (*Dendrolagus goodfellowi*)] (Reddacliff et al., 1997). However, the first and only molecular report of EMCV in felids was in three tigers kept at a semi-captive facility in China (Liu et al., 2013). One tiger was found dead, and two days later, another two individuals exhibited lethargy, depression, and anorexia that led to death despite antibiotic therapy for a week (Liu et al., 2013). EMCV was identified by RT-PCR and the potential source of infection was a nearby wild boar farm, since no rodents were found near the felids' enclosure (Liu et al., 2013). This report is important because it demonstrates that felids are also susceptible hosts to EMCV, which raises the question of

whether this virus has reached neotropical felids as well, especially those kept under human care or in zoo facilities.

3.3 *Rotavirus*

Rotaviruses are positive-sense double-stranded RNA viruses from the subfamily *Sedoreovirina* (Family *Reoviridae*) (Attoui et al., 2009). They were named due to their peculiar morphology, which resembles a wheel when visualized under electron microscopy (Lestari et al., 2023). Rotaviruses have a triple-layered capsid with no spicules, approximately 100 nm diameter, and a genome subdivided in 11 segments (Squires, 2014). Segments VP1-8 encodes structural proteins and segments NSP1-6 encodes non-structural proteins (Attoui et al., 2009). Proteins expressed by VP4 and VP7 are the most external and, therefore, probably more susceptible to mutations (Attoui et al., 2009). To further classify *Rotavirus A* (RVA) genotypes, molecular analysis of all the 11 segments is necessary (Ghosh & Kobayashi, 2014). RVA classification follows the structure Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, where x corresponds to the genotype number and each letter to one of the respective segments VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 (Ghosh & Kobayashi, 2014). This new classification system allows researchers to establish with greater precision the genetic relationships between RVA of animal and human origin (Martella et al., 2011). Over 50 different genotypes have been suggested, revealing a complex evolution of this viral agent with several recombination events between RVA of animal and human origin (Squires, 2014; Ng et al., 2014; Gabriel et al., 2023; Lestari et al., 2023). Although RVA is the most common *Rotavirus* (RV), it can be classified into nine species, *Rotavirus A-I* (Olaimat et al., 2024). All of these are capable of crossing species barriers and infecting humans, wild, and domestic animals (Nakagomi & Nakagomi, 1989; Ghosh & Kobayashi, 2014; Phan et al., 2017).

The primary transmission pathway of RV is the fecal-oral route, either through direct contact or contaminated fomites (Attoui et al., 2009; Gabriel et al., 2023). After oral ingestion of viral particles, RV replicates and compromises the villi of small intestine enterocytes, resulting in malabsorption of nutrients, fluids, and electrolytes (Olaimat et al., 2024). RV pathology may be primary or associated to other etiological

agents, presenting watery diarrhea, weight loss and dehydration as the main clinical manifestations in animals, especially in young and immunosuppressed individuals (Ng et al., 2014; Almeida et al., 2018; Gabriel et al., 2023). Nonetheless, RV has also been identified in fecal samples of healthy asymptomatic animals (Marshall et al., 1987; de Barros et al., 2018).

Most adult domestic cats have serological evidence of previous contact with RV, probably due to the queen's behavior of cleaning the perineal area of her kittens to stimulate defecation (Squires, 2014; German et al., 2015). This maternal coprophagy may reduce the risk of active infections in kittens via "oral vaccination" by the queen, which increases the kittens' anti-RV antibodies and thus decreases the chances of diarrhea (German et al., 2015). However, these kittens may still act as infection multiplicators even without clinical manifestations (German et al., 2015). Consequently, domestic felids are considered common dispersers of enteric viruses (Ng et al., 2014).

Although there is still no clear association between RV infection and diarrhea in felids, this is not the case in humans (Gabriel et al., 2023). In humans, RV is considered the main agent responsible for diarrhea outbreaks in children and is frequently associated with high mortality and morbidity worldwide (Olivares et al., 2020; Gabriel et al., 2023). Some authors hypothesize that domestic cats may act as a source of infection for humans, not only due to RV's ability to cross species barriers but also due to phylogenetic closeness between felid and human RVs, with several recombination events in their evolutionary history (Marshall et al., 1987; De Grazia et al., 2010; Martella et al., 2011; Squires, 2014). Nevertheless, there is a report of a child, who owned a cat as a pet, infected by a RV highly similar to those isolated in these felids (Nakagomi & Nakagomi, 1989).

Besides infection through contact with contaminated animals, RV viral particles are stable even in hostile environments and they are able to persist in water and food (Olaimat et al., 2024). For instance, both RVs of animal and human origin were detected in sewage water samples worldwide at relatively high rates, with higher occurrences detected in less economically developed countries (Miura et al., 2022; Gabriel et al., 2023; Hoque et al., 2023; Zamora-Figueroa et al., 2024). In Brazil, RVs of both animal and human origin have been isolated in fecal samples of newborn babies with diarrhea within the Amazon Forest region and in feces of domestic felids from the same area (de Barros et al., 2018; Maestri et al., 2021). In Southeast Brazil, different genotypes of RV were also detected in the feces of children (Santos et al., 2001).

Prevention of RV infection involves biosecurity measures, but vaccination of children in endemic areas, including Latin America, is also an option (Maestri et al., 2012). It is important to highlight that vaccines against RV may mitigate the disease but cannot completely prevent infection, especially because RVs may evade vaccine-induced immune pressure and recombine in new resistant genotypes (known as escape mutants) (Maestri et al., 2012; Zamora-Figueroa et al., 2024). In this context, long-term molecular surveillance is crucial to monitor any changes in viral genotypes and to manage potential emerging infections (de Barros et al., 2018; Zamora-Figueroa et al., 2024).

3.3.1 *Rotavirus* in wild felids

The only molecular report of *Rotavirus* in wild felids is on free-ranging snow leopards, where four out of seven tested animals were RV-positive by molecular detection in rectal swab samples (Johansson et al., 2020). However, the RV identified in this study was phylogenetically more closely related to RVs previously detected in humans and bovids, suggesting the possibility of either a novel RV strain, a spillover of an already known RV, or even the ingestion of an infected prey (Johansson et al., 2020).

It is also worth mentioning a serologic report of RVA in two young wild felids from the Brazilian Amazon, one ocelot and one oncilla (Gabriel et al., 2023). Both cubs were free-ranging admitted to a rehabilitation center with clinical manifestations including postprandial vomiting, rapidly progressive cachexia, dehydration, and watery yellowish diarrhea with a fetid odor (Gabriel et al., 2023). Despite pharmacological treatment, both animals died within less than 15 days (Gabriel et al., 2023). They did not yield positive molecular results for RVA, only serological evidence of a possible previous exposure to this virus. Thus, there is a lack of knowledge on whether RVA is circulating among wild felids in Brazil or not, which highlights the need of molecular RVA research on these species.

MATERIAL & METHODS

4 MATERIAL AND METHODS

4.1 Permissions

This study was approved by the Animal Ethics Committee of the School of Veterinary Medicine and Animal Science of the University of the State of São Paulo “Júlio de Mesquita Filho” (FMVZ – UNESP Botucatu) under protocol 0239/2022. Authorization for collection and transportation of biological materials from non-domestic felids was granted by the Biodiversity Authorization and Information System (SISBio), identified by numbers 84350 and 76428. Finally, this study was also registered on National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration code A5A3124.

4.2 Sampling area

Retrospective and prospective frozen samples were analyzed in this study, including animals sampled from 2016 to 2024. All samples were from wild felids, including free-ranging and animals under human care, with or without clinical manifestations, live and deceased. Samples were provided by affiliated institutions, as well as biological and epidemiological data and the animal’s clinical history when available.

Prospective samples of live animals were collected at three different institutions, comprising the great biomes of Amazon Forest, Atlantic Forest and Caatinga, as shown in Figure 1. Two jaguars and one cougar were sampled during jaguar scientific captures promoted by the National Center for Research and Conservation of Carnivorous Mammals (CENAP) at two different Conservation Unities, both ruled by Chico Mendes Institute for Biodiversity Conservation (ICMBio). One of them is the National Park Serra da Capivara (PNSC – PI), located in an area of 130,000 hectares under the coordinates 8º 25’ S 42º 20’ W, within a semi-arid Caatinga biome. This park features canyon

formations, low vegetation that loses its leaves during dry seasons, and underground caves, which are flooded during the rainy season (November to April), when there is emergence of small lagoons surrounded by greener and taller vegetation (ICMBio, 2019).

The other Conservation Unity is the Gurupi Biological Reserve (ReBio Gurupi – MA). It has an approximated area of 27,000 hectares and it is in a transition zone between the humid climate of Northern Brazil and the semi-arid climate of Northeast (3º 48’ S 45º 46’ W). The Reserve is marked by high vegetation and frequent rains, but it is in an area of the Eastern Amazon extremely deforested and fragmented, with occupations by indigenous populations on the surroundings (ICMBio, 1999). This is also a location of reportedly illegal wood overexploitation and hunting of several wild animals, including felids.

All animals kept under human care analyzed in this project are from a zoo in São Paulo state located in an area of transition between the Atlantic Forest and Cerrado biomes. The Atlantic Forest is the most fragmented Brazilian biome, with frequent rains and tall, arboreal and relatively dense remaining vegetation. Cerrado, on the other hand, is characterized by open areas, warmer temperatures and vegetations marked by crooked trunks, shrubs and grasses. The transition areas of these biomes have characteristics of both.

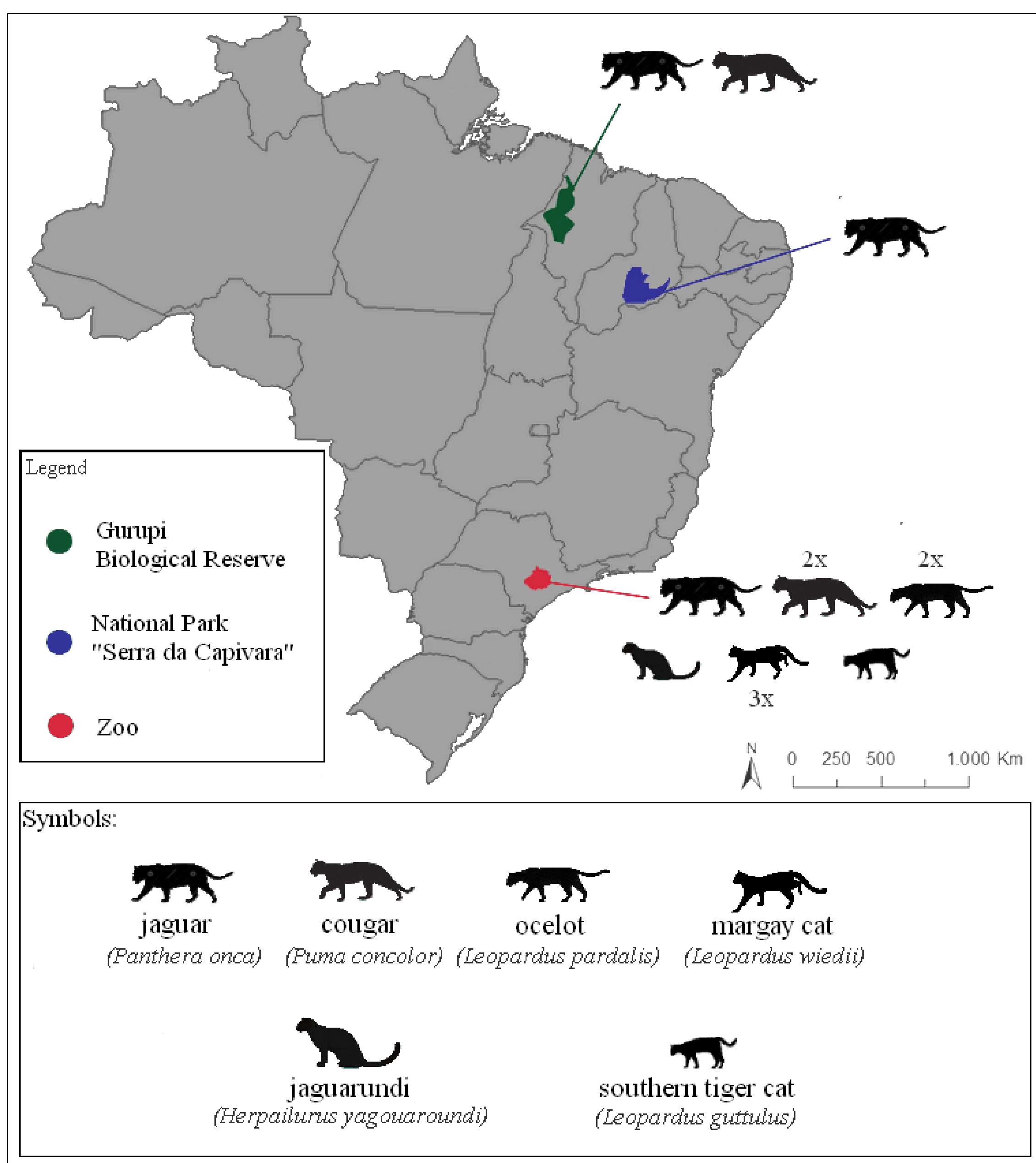

Figure 1. Live felids analyzed in this project and their places of origin and sampling collection.

Roadkill felids were also analyzed in this project. They were found on the side of different highways in Southeast Brazil, as shown in Figure 2. Most of them were located within the state of São Paulo, in areas of Atlantic Forest, Cerrado and transition zones between them. Feline carcasses from coastal regions of São Paulo and Rio de Janeiro were also sampled. Such locations are characterized by restingas, a grassy type of vegetation specifically adapted to sandy soil and humid climate influenced by the sea. Finally, ocelots from areas of Araucaria vegetation were sampled. This type of vegetation is characterized mainly by large dioecious trees and is more commonly found between the states of São Paulo and Paraná.

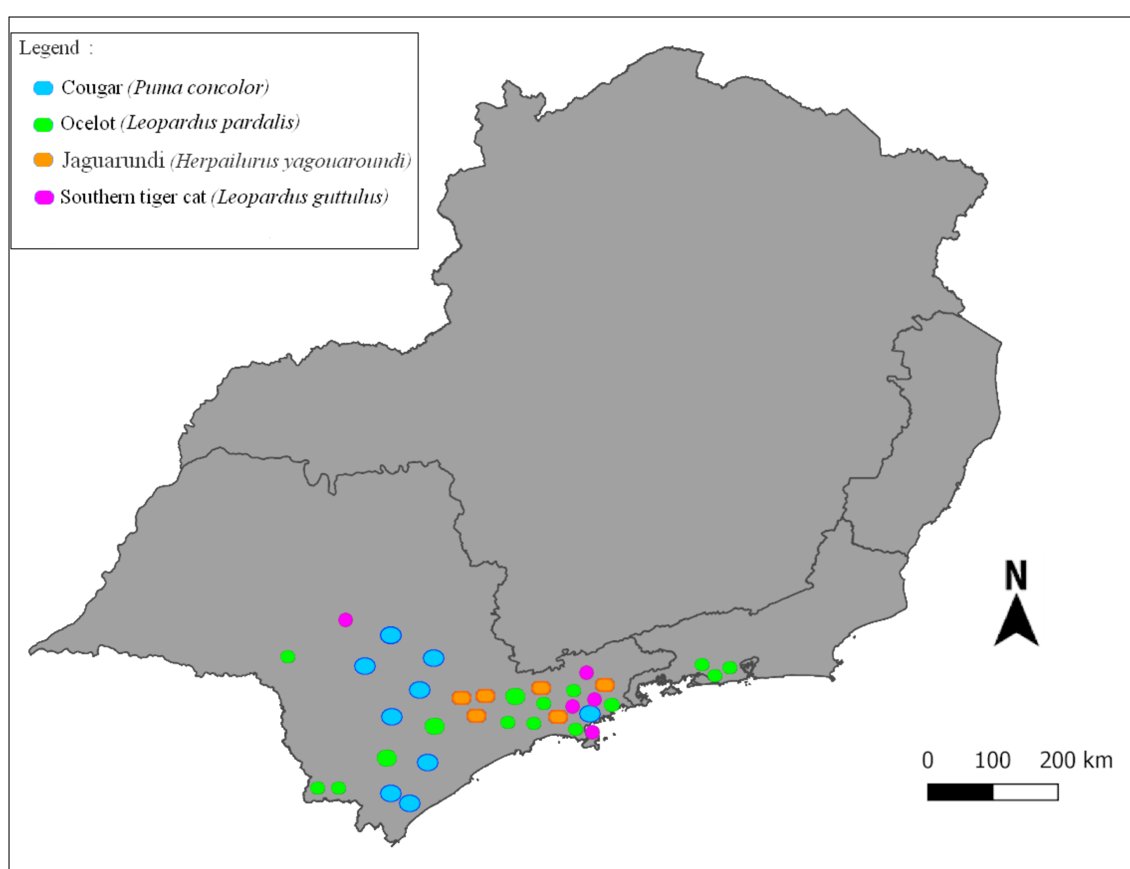

Figure 2. Areas where the roadkill felids analyzed in this project were found. The main highways where animals were found included BR 116, SP 99, SP 127 and SP 270.

4.3 Samples

Samples obtained from live animals were collected after they were previously chemically restrained, following anesthetic protocols established by the institution responsible for each procedure. After collection, samples were immediately frozen until they were transported to the Laboratory of Wildlife Comparative Pathology (LAPCOM), at FMVZ of the University of São Paulo (USP), where they were stored at -80ºC. Regarding the deceased animals, these samples were previously used in studies conducted at LAPCOM (Navas-Suárez, 2022; Lugo, 2024). All carcasses were frozen until transported to LAPCOM where they underwent a standardized necropsy procedure, with the collection of samples for histopathology (formalin 10%) and molecular analysis (frozen at -80ºC).

4.3.1 *Blood*

Blood samples were collected and stored in tubes with ethylenediaminetetraacetic (EDTA). The adopted collection protocols (e.g., puncture vessel, needle size, prior asepsis method) were previously established by the institution responsible for the animal. All samples were frozen immediately after collection until they were transported to LAPCOM, where they were stored at -80ºC.

4.3.2 *Swab, tissues and feces*

Oral, nasal, rectal, vaginal, and urogenital swab samples were collected. All the cotton swabs were rotated against the mucous wall and then immediately immersed in 2 ml RNase Free cryotubes containing 300 µL of 10 mmol/L phosphate buffered saline (PBS). These were frozen afterward until transported to LAPCOM, where they were stored at -80ºC.

Available tissues from roadkill felids, including brain, cerebellum, lung, heart, spleen, liver, mesenteric and submandibular lymph nodes, kidney, tonsils, skeletal muscles, and feces available in the LAPCOM tissue bank and frozen at -80ºC were analyzed. Whenever available, more than one tissue sample per animal was studied.

4.4 Molecular analysis

4.4.1 *RNA extraction*

Total RNA extraction from EDTA blood samples was performed using the commercial QIAmp RNA Blood Mini Kit (Qiagen, Valencia, CA, EUA) following the manufacturer's instructions. The total RNA extracted was later stored at -80ºC freezers until further processing.

Tissues and fecal samples were aliquoted in 3 mm^3 pieces using sterilized tongs, Petri dish, and scalpel. Aliquots were then macerated with 400 µL of RLT Buffer from Qiagen RNeasy Mini Kit (Valencia, CA, USA) using Bio-Gen Pro200 homogenizer (Pro Scientific, Oxford, USA). After maceration, total RNA was extracted using the same RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Total RNA from swab samples was extracted using the same kit. All total RNA extractions were later stored at -80ºC before further processing.

All extraction procedures were conducted at LAPCOM using a Z 32 HK centrifuge (HERMLE Labortechnik GmbH, Alemanha). In all extractions 100 µL of RNase-free water (Qiagen, Valencia, CA, EUA) was used as negative control. Total RNA extractions were quantified using BioTek EPOCH equipment (Agilent, Santa Clara, CA, EUA) before being submitted to further molecular analysis.

Initial screening was performed with target tissues depending on the selected virus pathogenicity. In case of positive animals, their other available biological samples were submitted to the same molecular reaction.

4.4.2 *Astrovirus RT-PCR*

AstV detection via RT-PCR was performed by targeting gene ORF1b using a nested protocol previously published (Atkins et al., 2009; Rivera et al., 2010; Lawler et al., 2018). For protocol standardization and validation, positive control samples were utilized. The positive control samples of HAstVs 1-8 were kindly provided by the biological sample repository of the Comparative and Environmental Virology Laboratory (LVCA) at Oswaldo Cruz Institute (IOC), Rio de Janeiro (RJ). This laboratory is part of the Ministry of Health's structure and plays a crucial role in the surveillance of acute gastroenteritis outbreaks in humans in Brazil.

The first round of the nested RT-PCR was made with OneStep Enzyme (Qiagen, Valencia, CA, EUA) according to standard protocol, using 4 µL of each sample

and 20 µM primers Astr4380F and Astr4811R (Table 1). RNase-free water was added to reach a total volume reaction of 25 µL. This mixture was amplified in C1000 Touch Thermal Cycler (Bio-Rad, CA, EUA) with the following conditions: initial denaturation at 50ºC for 30’, reverse transcriptase reaction at 95ºC for 15’, 36 cycles of denaturation at 94ºC for 30’’; annealing at 47ºC for 30’’; extension at 72ºC for 30’’, and a final extension at 72ºC for 7’. The second round of PCR was made using 4 µL of the first RT-PCR product, 20 µM primers Astr4574F and Astr4722R (Table 1) and polymerase enzyme TaqPlatinum (Invitrogen, Thermo Fisher, MA, USA). RNase-free water was added until a total volume reaction of 25 µL. This mixture was amplified with the following conditions: initial denaturation at 94ºC for 5’, followed by 36 cycles of denaturation at 94ºC for 30’’; annealing at 47ºC for 30’’; extension at 72ºC for 30’’, and a final extension at 72ºC for 7’. RNase Free Water (Qiagen, Valencia, CA, EUA) was used as negative control.

Amplified products were resolved in electrophoresis 1,5% agarose gel stained with SYBR Safe DNA (Invitrogen, Thermo Fisher, MA, EUA), and applied with BlueJuice Gel Loading Buffer 1X (Invitrogen, Thermo Fisher, MA, EUA).

Table 1. AstV primers targeting gene ORF1b used in this nested RT-PCR protocol. Primers Astr4380F and Astr4811R were used in the first round to amplify a 430 bp fragment, while primers Astr4574F and Astr4722R were used in the second round to amplify a 150 bp fragment.

Primer	Nucleotid sequence (5’-3’)	Reference
Astr4380F	GAYTGGRCNCGNTWYGATGGNACIAT	Atkins et al., 2009
Astr4811R	GGYTTNACCCACATNCCAAA	
Astr4574F	GGNAAYCCMTCWGGICA	
Astr4722R	ARNCKRTCATCNCCATA	

Amplified 150 pb fragments were purified using ExoSAP-IT enzyme (Affymetrix, CA, EUA), according to the fabricant’s instructions. Purified products were then submitted to Sanger sequencing in both directions, with the same two primers used in the second round of PCR diluted at 5 µM.

4.4.3 *Cardiovirus RT-qPCR*

EMCV was investigated via RT-qPCR. The protocol was performed with Kapa SYBR Fast Enzyme, 50X RT Kapa enzyme and ROX Reference Dye High, following concentrations suggested by the fabricant (Applied Biosystems, Thermo Fisher, MA, USA). The selected 30 µM primers EMCV-P1 and EMCV-P2 target a 286 pb fragment of gene 3D (Table 2). Each reaction was made with 4 µL of sample. RNase Free Water (Qiagen, Valencia, CA, EUA) was added until reaching a final volume reaction of 20 µL. Reaction times followed were: reverse transcriptase reaction at 42ºC for 5', initial denaturation at 95ºC for 3', followed by 45 cycles of denaturation at 95ºC for 3''; annealing at 60ºC for 30''; extension at 95ºC for 15'', melting at 60ºC for 1' and a final extension step at 95ºC for 15''. The amplification was performed in a StepOnePlus™ System (Applied Biosystems, Thermo Fisher, MA, USA).]

Table 2. Primers used in this RT-qPCR protocol to detect a 286 bp fragment of EMCV's gene 3D.

Primer	Nucleotid sequences 5'-3'	Reference
EMCV-P1	CCCTACCTCACGGAATGGGGCAAAG	Vanderhallen e Koenen, 1998
EMCV-P2	GGTGAGAGCAAGCCTCGCAAAGACAG	

Positive control samples were kindly provided by Dr. David Driemeier, from the Veterinary Pathology Sector of the Federal University of Rio Grande do Sul (UFRGS), which was later serially diluted with RNase-free water. The latter was also used as a negative control in all reactions. All samples were diluted in RNase-free water to a 1:10 proportion before submitting them to RT-qPCR. Samples with values of Cycles threshold (Ct) lower than 35 and melting temperature (Tm) higher than 80ºC were considered suspected and were then purified with ExoSAP-IT enzyme (Affymetrix, CA, EUA), following the same conditions previously mentioned. Purified samples were finally sent to Sanger sequencing in both directions, also following the same conditions previously mentioned.

4.4.4 *Rotavirus RT-qPCR*

Total RNA extractions were tested for RVA at the LVCA (IOC, RJ) using a RT-qPCR protocol previously published (Zeng et al., 2008). The reaction was made with a RT SIII/Taq enzyme and 10 µM probe and primers targeting gene NSP3, as shown in Table 3. Five µL of each sample was added to the mix reaction and RNase Free Water (Qiagen, Valencia, CA, EUA) was used to complete a total volume reaction of 25 µL. The amplification temperatures were as follows: transcriptase reverse reaction at 55ºC for 30', initial denaturation at 95ºC for 10', followed by 40 cycles of denaturation at 95ºC for 15''; hybridization and extension at 60ºC for 1'. The reaction was performed in QuantStudio 3 (Applied Biosystems, Thermo Fisher, MA, USA) thermocycler. Amplification plots of fluorescence intensities were used at different 10-fold dilutions, such as 10^3, 10^4, and 10^6. A positive control was also used as reference, as well as RNase Free Water (Qiagen, Valencia, CA, EUA) as negative control.

Table 3. Primers and probe used in this RT-qPCR protocol to detect a 80 bp fragment of the RVA NSP3 gene.

Primers and probe	Nucleotid sequence (5'-3')	Reference
NSP3F	ACCATCTWCACRTRACCCTCTATGAG[a]	Zeng et al., 2008
NSP3R	GGTCACATAACGCCCCTATAGC	
TaqMan Probe	FAM-AGTTAAAAGCTAACACTGTCAAA-MGB	

4.5 Phylogenetic analysis

Obtained sequences were assembled in MEGA 7.0 program (Kumar et al., 2016) using ClustalW alignment and compared with those available in GenBank by BLASTn search (https://blas.ncbi.nlm.nih.gov/Blas_t.cgi). Nucleotide genetic distances to the closest sequences were calculated based on p-distance after editing out the primers. Finally, nucleotide maximum likelihood phylogenetic tree was constructed using MEGA 7.0 with a bootstrap value of 1,000 replicants and a Neighbor-Joining model for AstV

ORF1b gene (150 bp). The evolutionary model selected was p-distance, based mostly on other reports (Sun et al., 2014). All bootstrap frequency values less than 70 were omitted.

4.6 Histopathology

In positive cases for any of the viral agents, histopathological analysis was conducted on formalin-fixed samples, when available. Formalin-fixed tissues were embedded in paraffin until sectioned in portions of 3-5 μm in thickness and placed into plastic cassettes for routine histopathological processing. Finally, the slides were stained with hematoxylin and eosin (HE).

The histopathological analysis followed the fundamental levels of morphological diagnosis proposed by national organizations, such as the Brazilian Association of Veterinary Pathology, and international organizations, such as the American College of Veterinary Pathologists and the European College of Veterinary Pathologists. Thus, the lesions were described referencing the affected organ, the type of lesion, its distribution, intensity, and temporality.

RESULTS

5 RESULTS

5.1 Samples

A total of 50 wild felids were analyzed in this project, including animals from six different species: jaguar (n = 3), cougar (n = 12), ocelot (n = 19), jaguarundi (n = 7), margay cat (n = 3) and southern tiger cat (n = 6). Available epidemiological, biological, and sampling data on all animals are included in Appendix 1.

RNA extraction was performed in the following samples: EDTA blood (n = 13), oral swab (n = 14), nasal swab (n = 13), rectal swab (n = 14), vaginal swab (n = 9), urogenital swab (n = 1), brain (n = 23), cerebellum (n = 7), lung (n = 27), heart (n = 29), spleen (n = 20), liver (n = 25), mesenteric lymph node (n = 23), submandibular lymph node (n = 2), kidney (n = 27), tonsils (n = 9), skeletal muscle (n = 13), stomach (n = 1), adrenal gland (n = 1), trachea (n = 1) and feces (n = 7) (Appendix 1), totalizing 279 extracted samples.

Not all animals were tested for all viruses. Individuals were tested by each virus according to available biological samples, as shown in Table 4. Of the total 50 wild felids studied, 43 were tested for all three viruses.

Table 4. Distribution of the animals tested for each of the selected RNA viruses, according to ID, species, age group, sex, and origin.

A: Adult; Y: Young; C: Cub; M: Male; F: Female; AstV: Astrovirus; EMCV: Encephalomyocarditis Virus; RVA: Rotavirus A.

*: Roadkill animals. Cities where they were found on the highways and the highways codes are noted, respectively.

ID	**Species**	**Age Class**	**Sex**	**Origin**	**AstV**	**EMCV**	**RVA**
MC4890	*P. concolor*	A	M	Salto de Pirapora (SP)	X	X	
MC9440	*L. pardalis*	A	M	Zoo	X	X	X
MC3794	*L. wiedii*	A	F	Zoo	X	X	X

MC4769	*L. pardalis*	A	F	Zoo	X	X	X
MC4566	*L. guttulus*	A	F	Zoo	X	X	X
MC9574	*P. concolor*	A	M	Zoo	X	X	X
MC2557	*L. wiedii*	A	M	Zoo	X	X	X
MC3910	*L. wiedii*	A	M	Zoo	X	X	X
MC8105	*P. concolor*	Y	F	Zoo	X	X	X
CAD272.283	*L. guttulus*	C	F	Botucatu (SP)	X		X
bPon543	*P. onca*	A	M	PNSC (PI)	X		X
VIT	*P. onca*	A	F	Zoo	X	X	X
MC3490	*H. yagouaroundi*	A	F	Zoo	X	X	X
BANG	*P. concolor*	Y	F	Botucatu (SP)	X	X	X
ML	*P. concolor*	Y	F	ReBio Gurupi (MA)	X	X	X
bPon547	*P. onca*	A	M	ReBio Gurupi (MA)	X		X
CS-001	*L. pardalis*	A	M	*Caraguatatuba (SP) – SP99	X	X	X
CS-002	*L. pardalis*	Y	F	*Paraibuna (SP) – SP99	X	X	X
CS-009	*P. concolor*	Y	M	*Cajati (SP) – BR116	X	X	X
CS-012	*L. guttulus*	Y	F	*Caraguatatuba (SP) – SP99	X	X	X
CS-014	*H. yagouaroundi*	A	M	*Paraibuna (SP) – SP99	X	X	X
CS-016	*L. guttulus*	A	M	*Caraguatatuba (SP) – SP310	X	X	X
CS-018	*P. concolor*	A	F	*Corumbatai (SP) – SP310	X	X	X
CS-019	*L. pardalis*	A	M	*Caraguatatuba (SP) – SP99	X	X	X
CS-025	*L. pardalis*	A	M	*Caraguatatuba (SP) – SP310	X	X	X
CS-026	*L. pardalis*	Y	M	*Caraguatatuba (SP) – SP310	X	X	X
CS-027	*L. pardalis*	A	F	*Caraguatatuba (SP) – SP310	X	X	X
CS-031	*P. concolor*	A	F	*S. Miguel Arcanjo (SP) – SP331	X	X	X
RK-032	*L. pardalis*	Y	M	*Paraibuna (SP) – SP99	X	X	X
RK-034	*P. concolor*	A	M	*Caraguatatuba (SP) – SP99	X	X	X
RK-039	*L. guttulus*	Y	F	*Caraguatatuba (SP) – SP99	X	X	X
RK-042	*P. concolor*	Y	F	*Piracicaba (SP) – SP127	X	X	X
RK-062	*H. yagouaroundi*	Y	M	*Jambeiro (SP) – SP99	X	X	X
RK-066	*L. pardalis*	A	M	*Caraguatatuba (SP) – SP99	X	X	X
RK-067	*L. pardalis*	Y	M	*Miracatu (SP) – BR116	X	X	
RK-088	*H. yagouaroundi*	A	M	*Atibaia (SP) – BR381	X	X	X
RK-089	*H. yagouaroundi*	Y	M	*Atibaia (SP) – BR381	X	X	X
RK-104	*H. yagouaroundi*	A	M	*Atibaia (SP) – BR381		X	
RK-175	*L. pardalis*	A	F	*Caraguatatuba (SP) – SP99	X	X	X
RK-205	*H. yagouaroundi*	A	M	*Paraibuna (SP) – SP99	X	X	X

RK-206	*P. concolor*	Y	F	*Registro (SP) – BR116	X	X	X
RK-263	*L. pardalis*	Y	M	*Ipaussu (SP) – SP270	X	X	X
RK-273	*L. pardalis*	Y	F	*Duque de Caxias (RJ) – BR116	X	X	X
RK-276	*L. pardalis*	A	M	*Cajati (SP) – BR116	X	X	X
RK-278	*L. pardalis*	A	M	*Duque de Caxias (RJ) – BR116	X	X	X
RK-280	*P. concolor*	Y	M	*Cajati (SP) – BR116	X	X	X
RK-303	*L. guttulus*	Y	M	*Paraibuna (SP) – SP99	X	X	X
RK-310	*L. pardalis*	A	M	*Caraguatatuba (SP) – SP99		X	X
RK-400	*L. pardalis*	A	M	*Paraibuna (SP) – SP99	X	X	X
RK-414	*L. pardalis*	A	M	*Paraibuna (SP) – SP99	X	X	X

5.2 Astrovirus

Total RNA extracted from 48 felids was tested, with more than one sample per animal whenever target tissues were available (Table 5). Prioritized tissue samples for an initial AstV screening included: EDTA blood (n = 13), rectal swab (n = 14), oral swab (n = 14), spleen (n = 20), mesenteric lymph node (n = 20), intestine (n = 23), brain (n = 26), and feces (n = 6), totalizing 136 tested samples. In positive cases, other tissues available from the same animal were submitted to the same RT-PCR.

Table 5. Wild felids and their respective target tissues selected for initial AstV screening.

A: Adults; Y: Young; FR: Free Raging; HU: kept under human care; BL: Blood; RS: Rectal Swab; OS: Oral Swab; SP: Spleen; ML: Mesenteric Lymph node; IN: Intestines; BR: Brain; FE: Feces.

Species	**N**	**Males**		**Females**		**Origin**		**Samples (n = 136)**							
		A	**Y**	**A**	**Y**	**FR**	**HU**	**BL**	**RS**	**OS**	**SP**	**ML**	**IN**	**BR**	**FE**
P. concolor	12	3	2	2	5	10	2	5	4	4	5	6	6	6	1
L. pardalis	18	9	4	3	2	16	2	2	2	2	8	9	12	14	2
L. wiedii	3	2	0	1	0	0	3	3	3	3	0	0	0	0	0
L. guttulus	6	1	1	1	3	5	1	1	2	2	3	3	4	3	2

P. onca	3	2	0	1	0	2	1	1	2	2	0	0	0	0	0
H. yagouaroundi	6	3	2	1	0	5	1	1	1	1	4	2	1	3	1
Total	**48**	20	9	9	10	38	10	13	14	14	20	20	23	26	6

In total, 136 samples were initially screened for AstV by RT-PCR, with one positive result (0.7%). This sample was a rectal swab from a captive southern tiger cat, representing 7.1% of all rectal swabs tested (1/14). Thus, one of 48 tested wild felids was positive for AstV detection via RT-PCR, with an occurrence rate of 2.1% for all species tested and 16.6% (1/6) specifically for this species. The positive animal was a female adult maintained at a zoo in São Paulo state, representing 10% (1/10) of all zoo wild felids tested. The animal was considered healthy upon clinical examination on the same date of sample collection. Upon the positive result on rectal swab analysis, all other samples available from this animal (i.e., EDTA blood, oral swab, nasal swab, and vaginal swab) were submitted to pan-astrovirus RT-PCR protocol, with negative results.

The retrieved AstV sequence was 150 bp long and presented 90.48% nucleotide identity (Query Cover [QC]: 98%) with the closest available sequences in GenBank: Chicken Astroviruses from the United Kingdom (accession numbers JN582327 and EU668998) and Iran (MN871901). On the phylogram, the retrieved sequence clustered closer to chicken astroviruses than to feline astroviruses (Figure 3).

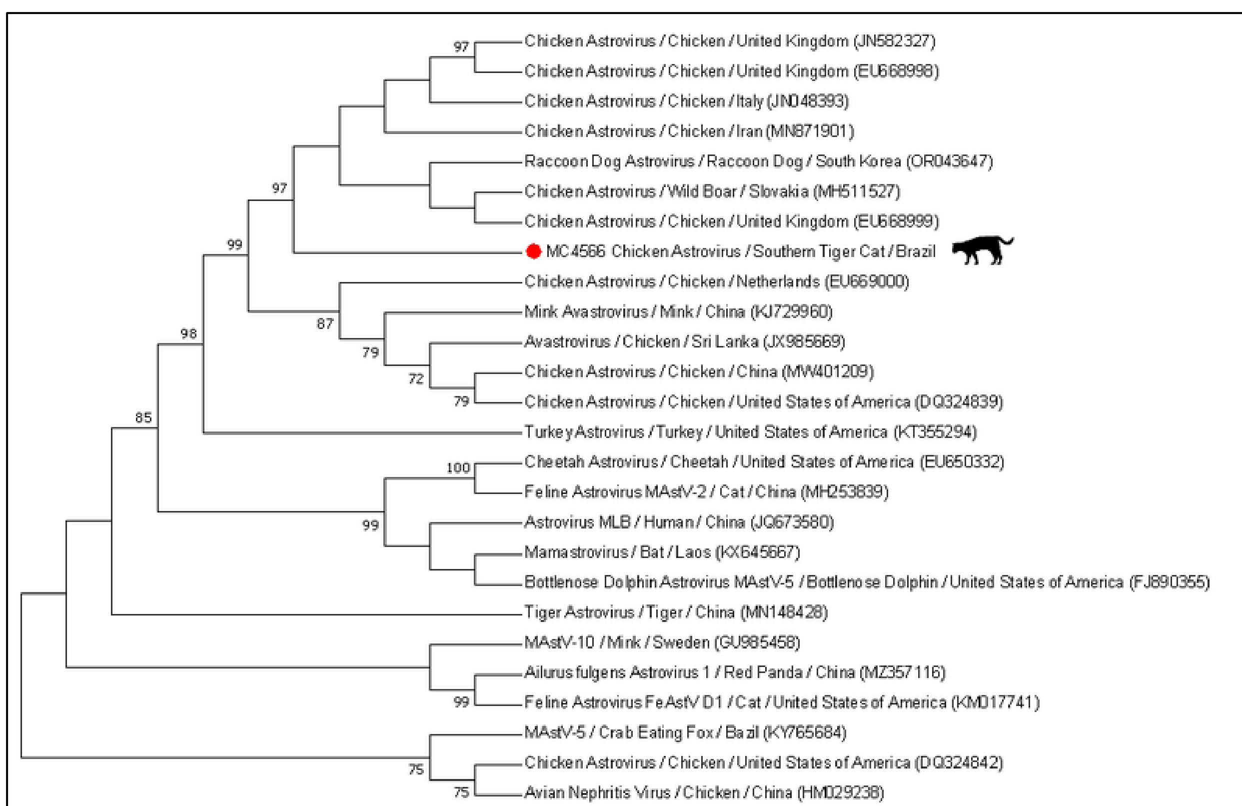

Figure 3. Phylogenetic analysis of partial (150 bp) AstV ORF1b gene. Phylogenetic tree was generated in MEGA7 using Neighbor-Joining method with p-distance and bootstrapped with 1,000 replications. Values under 70% were omitted. Sequence from southern tiger cat clustered closer to Chicken Astrovirus.

5.3 Cardiovirus

Animals and their selected target tissues for initial screening for EMCV are shown in Table 6. The following samples were prioritized: EDTA blood (n = 13), brain (n = 25), heart (n = 28), lungs (n = 26), liver (n = 24), and spleen (n = 18), totalizing 134 samples.

Despite testing 134 biological samples from 47 different wild Brazilian felids, no animal was positive to EMCV by the broad-spectrum RT-PCR described herein.

Table 6. Wild felids and their respective target tissues selected for initial EMCV screening.

A: Adults; Y: Young; FR: Free Raging; HU: kept under human care; BL: Blood; BR: Brain; HE: Heart; LU: Lungs; SP: Spleen; LI: Liver

Species	**N**	**Males**		**Females**		**Origin**		**Samples (n = 134)**					
		A	**Y**	**A**	**Y**	**FR**	**HU**	**BL**	**BR**	**HE**	**LU**	**SP**	**LI**
P. concolor	12	3	2	2	5	10	2	5	6	5	7	4	4
L. pardalis	19	10	4	3	2	17	2	2	13	13	12	7	12
L. wiedii	3	2	0	1	0	0	3	3	0	0	0	0	0
L. guttulus	5	1	1	1	2	4	1	1	3	4	3	3	3
P. onca	1	0	0	1	0	0	1	1	0	0	0	0	0
H. yagouaroundi	7	4	2	1	0	6	1	1	3	6	4	4	5
Total	**47**	20	9	9	9	37	10	13	25	28	26	18	24

5.4 Rotavirus

A total of 47 wild felids were analyzed, with 58 total RNA extractions initially screened for RVA (Table 7). The selected target tissues were oral swab (n = 1), rectal swab (n = 14), intestine (n = 23), feces (n = 7), mesenteric lymph node (n = 4), and liver (n = 9), totalizing 58 samples.

Table 7. Wild felids and their respective target tissues selected for initial RVA screening.

A: Adults; Y: Young; FR: Free Raging; HU: kept under human care; FE: Feces; RS: Rectal swab; OS: Oral swab; IN: Intestine; ML: Mesenteric Lymph node; LI: Liver.

Species	**N**	**Males**		**Females**		**Origin**		**Samples (n = 58)**					
		A	**Y**	**A**	**Y**	**FR**	**HU**	**FE**	**RS**	**OS**	**IN**	**ML**	**LI**
P. concolor	11	2	2	2	5	9	2	1	4	0	6	1	1
L. pardalis	18	10	3	3	2	16	2	3	2	0	12	2	4
L. wiedii	3	2	0	1	0	0	3	0	3	0	0	0	0
L. guttulus	6	1	1	1	3	5	1	2	2	0	4	0	0
P. onca	3	2	0	1	0	2	1	0	2	1	0	0	0
H. yagouaroundi	6	3	2	1	0	5	1	1	1	0	1	1	4
Total	**47**	20	8	9	10	37	10	7	14	1	23	4	9

The positive controls used in this reaction as well as the amplification plots of fluorescence intensities (10^3, 10^4 and 10^6) Ct values ranged between 24.07 and 32.98. Of all 58 analyzed samples, one young female cougar small intestine had a Ct = 28.91, thus considered positive (1.7%). Regarding tested intestines, it represents an occurrence rate of 4.3% (1/23).

Upon this result, other available tissues of the same animal were tested (i.e., trachea, heart, stomach, adrenal gland and submandibular lymph node) under identical conditions. The trachea was also considered positive when compared to the positive controls and amplification plots, even though it had a higher Ct value (Ct = 34.5) (Figure 4).

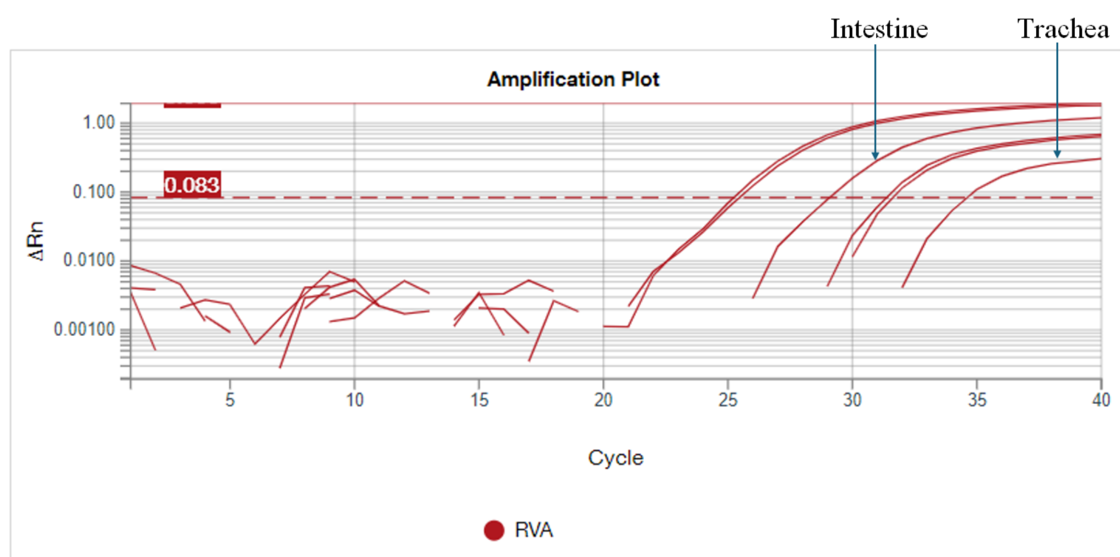

Figure 4. Amplification plots of intestine (Ct = 28.91) and trachea (Ct = 34.5) from the same young female cougar in comparison to the amplification plots of positive controls.

Of all 47 wild felids analyzed for RVA detection, one was positive (2.1%). Regarding species, 9% of all tested cougars were RVA-PCR positive (1/11), representing 2.7% (1/37) of all free-living wild felids tested and 3.1% of all roadkill felids (1/32). This animal was found in SP127 highway within São Paulo state, close to Piracicaba city. Histopathology slides of small intestine were available and upon analysis revealed infection by parasites, but further diagnosis was not possible due to autolysis degree.

DISCUSSION

6 DISCUSSION

Although astrovirus, cardiovirus and rotavirus have been previously reported in wild felids (Atkins et al., 2009; Liu et al., 2013; Zhang et al., 2019; Johansson et al., 2020), this is the first molecular screening for these infectious agents in American species. Four different genera were analyzed, comprising six different species that are currently facing populational decrease and at least one (i.e., the southern tiger cat) is classified as vulnerable to extinction (IUCN, 2024). In the present study, rectal swab of one specimen of this vulnerable cat was positive for AstV, representing 2.1% of all tested animals (1/48). This is a low occurrence rate when compared to AstV detected in domestic cat feces worldwide: 32.8% (40/122) in United States, 23.4% (46/197) in China, 21.1% (43/204) in Japan, 7.3% (4/55) in Portugal and 4.8% (11/228) in Australia (Marshall et al., 1987; Ng et al., 2014; Lawler et al., 2018; Yi et al., 2018; Soma et al., 2020). Most of these reports identified FeAstV strains that were very divergent from the retrieved sequence reported herein, which clustered within *Avastrovirus* genera on the phylogram. Furthermore, this sequence was not similar to any FeAstV previously detected, whether in domestic or wild felids (Atkins et al., 2009; Zhang et al., 2019; Van Brussel et al., 2020).

This is not the first time an *Avastrovirus* has been identified in a mammalian host, as previously reported in domestic cats, minks, and raccoon dogs (Sun et al., 2014; Lawler et al., 2018; Chae et al., 2023). Considering that all those mentioned species are carnivores, the infection with *Avastrovirus* was potentially related to ingestion of an infected prey or contaminated food of avian origin (Sun et al., 2014; Lawler et al., 2018). In our study, apart from rectal swab, all other biological samples from the southern tiger cat were AstV negative, suggesting ingestion of contaminated/infected prey rather than a systemic infection. The same has been proposed by Chae et al. (2023) with raccoon dogs (*Nyctereutes procyonoides*) in South Korea, as they identified different AstV strains within the same host, each in different tissues; AstV isolated from brain were more closely related to Canine Astroviruses, while sequences retrieved from intestine samples were more similar to Chicken Astrovirus. The sequence from our report shared 87.07% nucleotide identity with Chicken Astrovirus isolated from raccoon dogs' intestines (GenBank Accession Number OR043647). On a similar note, Chicken Astrovirus has

also been detected in feces of minks presenting gastroenteritis in China (Sun et al., 2014), and their genomic sequence (GenBank Accession Number KJ729960) shared 81.42% nucleotide identity with the sequence retrieved in this study. Those minks were fed with chicken, which was considered the potential source of infection that resulted in gastroenteric clinical manifestations (Sun et al., 2014). In our case, the adult southern tiger cat was considered healthy upon clinical examination, not presenting any signs potentially related to AstV infection. Indeed, diarrhea associated with AstV is not commonly reported in this age class, regardless of species (Pérot, Lecuit & Eloit, 2017). Additional tissues were analyzed in this project based on previous detections on extraintestinal sites (i.e., brain, intestine, mesenteric lymph node and feces) in wild carnivores (Alves et al., 2018; Chae et al., 2023), but no positivity was detected apart from the rectal swab.

In this study, we retrieved a 150 bp sequence of AstV ORF1b gene that was separated into a monophyletic branch within *Avastrovirus* genera with 97 bootstrap value in our phylogram. According to ICTV, the classification of novel AstV is based on phylogenetic analysis of gene ORF2 (Wang et al., 2020). However, ORF1b (RdRp) is considered the most conserved gene of AstV, thus, more recommended as a target for pan AstV protocols (Atkins et al., 2009; Rivera et al., 2010), as performed herein. Considering we analyzed a small fragment of a very conserved gene, the similarities of our retrieved sequence to closest AstV sequences available in GenBank were considered low (90.48%). These findings suggest the retrieved sequence may represent a novel astrovirus species. Astroviruses have low host species fidelity and high mutation rates, enabling rapid evolution that allows interspecific transmission (Sun et al., 2014; Lawler et al., 2018), a possibility that cannot be ruled out in this study. Our findings warrant further knowledge on AstV by reporting a potential novel AstV as well as widening the host range of this RNA virus, with its detection on a vulnerable South American wild felid. Further molecular characterization of whole genome would help solve whether this is an AstV infecting the wild felid or its prey.

Despite screening six different species of wild felids and tissue types, testing a total of 134 samples, and representing four different Brazilian biomes (i.e., Amazon Forest, Atlantic Forest, Caatinga and Cerrado), cardiovirus RNA was not detected. Those findings could indicate a low occurrence of this virus in this group of carnivores or even an inefficiency of the established diagnostic protocol. However, the positive controls provided were accordingly detected and sequenced, validating the

selected protocol, which was used in most of the previous detections (Gris et al., 2023). Furthermore, the selected tissues for EMCV screening in this project were in accordance with the virus pathogeny, which allegedly has a vascular endothelial tropism and may be disseminated systematically through the actions of macrophages (Lipton, Kumar & Hertzler, 2007; Foglia et al., 2023). Nevertheless, other reports have molecularly identified EMCV-RNA in EDTA blood, brain, heart, lung, liver, and spleen of different animal species (Reddacliff et al., 1997; Wang et al., 2012; Liu et al., 2013; Cardeti et al., 2016; O'Connor et al., 2020; Romey et al., 2021; Gris et al., 2023). Therefore, it is more likely that this is rather a low occurrence virus amongst the tested wild felids than an inefficient diagnostic protocol. Most of previous EMCV reports in wildlife included animals kept at facilities with a previous history of rodent infections (Canelli et al., 2010; Cardetti et al., 2016; O'Connor et al., 2020), and, in contrast, our sampling of free-raging animals (40/50) was higher than zoo animals (10/50). Furthermore, EMCV is a lithic rapid virus that generally do not persist in hosts, inducing sudden death (Canelli et al., 2010; Carocci & Bakkali-Kassimi, 2012; Liu et al., 2013; Cardetti et al., 2016). Considering that all sampled zoo wild felids in this project were alive and healthy, the detection of EMCV-RNA was not expected (Reddacliff et al., 1997). Additionally, this virus has not yet been reported in other species of free-ranging animals apart from rodents and wild boars (Billinis, 2009; Ao, Xu & Duan, 2022; Foglia et al., 2023), which is in accordance with our findings.

Despite not being detected in American wild felids in the present study, it is known EMCV can infect wild Asian species, as it has been fatally reported in tigers (Liu et al., 2013; Ao, Xu & Duan, 2022). Serologic surveys were already performed in healthy caracal (*Caracal caracal*), lion, jaguar, and tiger, with positive results only in lion (Reddacliff et al., 1997). Consequently, there is limited knowledge on EMCV epidemiology in felids, evidencing a further need for molecular screening of this infectious pathogen, particularly within areas where EMCV has been identified previously, including Brazil and South America (Oberste et al., 2009; Czechowicz et al., 2011; Gris et al., 2023). Despite our negative results, we reinforce that EMCV should be considered as a differential diagnosis of fatal myocarditis in wildlife, especially mammalian species in zoos (Romey et al., 2021).

Regarding RVA, this study characterizes the first detection in a wild felid species from America. Samples of trachea and small intestine of one young female cougar were positive in this project, representing a lower RVA occurrence (2.1%) than those

reported on healthy domestic cat feces: 12.7% (7/55) in Portugal and 84% (32/38) in Brazilian Amazon (Ng et al., 2014; de Barros et al., 2018). RVA have been reported in other wild carnivores (i.e., raccoons, badgers, coyotes, civets, red foxes, skunks, wild dogs and bears); however genotyping constellation was not performed (Alarcón, Liotta & Miño, 2022). Only partial genotyping of RVA detected in rectal swabs from free-ranging snow leopards was performed (Johansson et al., 2020). However, authors mentioned there was a lack of available RVA sequences from wild felids to compare their newfound partial genotype (Johansson et al., 2020), highlighting the need for RVA molecular screening in other wild felids species as performed herein. Most domestic cats RVA are classified as combinations of genotypes G3P[3] or G3P[9] with evidences of possible recombination events involving other host species (Ghosh & Kobayashi, 2014). Reassortment and recombination events are frequent amongst RNA viruses and may result in novel genotypes that can be transmitted to new host species with unpredictable results (Alarcón, Liotta & Miño, 2022). Ongoing studies will determine the genotype constellation of the RVA detected in the cougar's small intestine and trachea.

The detection of RVA RNA in small intestine is in accordance with the virus known pathogeny, which infects mainly intestinal enterocytes (Dian et al., 2021). However, RVA has already been detected in extraintestinal tissues from children and other animal species, such as pigs and rats (Lynch et al., 2003; Crawford et al., 2006). It is suggested that RVA can be detected in any tissue with blood supply, since it can be transported through vascular system to more distant organs and potentially result in systemic clinical manifestation (e.g., fever) (Lynch et al., 2003; Dian et al., 2021; Gutiérrez et al., 2021). In this project, RVA was detected not only in small intestine but also in the trachea of the same individual. Similar reports found RVA-RNA in oropharyngeal and tracheal aspirates of humans with respiratory symptoms (Zhaori et al., 1991; Zheng et al., 1991; Taboada et al., 2014; Madi et al., 2018; Thi Kha Tu et al., 2020). Additionally, RVA-RNA was detected in the respiratory tract of children with acute gastroenteritis (Satter et al., 2024). It is suggested that gastroenteritis caused by RVA exacerbates peristaltic movements in the small intestine and may result in refluxes, explaining the RVA detection in respiratory tract of these children (Satter et al., 2024). On the other hand, RVA replication in respiratory tract has also been suggested (Crawford et al., 2006; Satter et al., 2024). Even though RVA was detected in both gastrointestinal and respiratory tracts of the same animal, it is not possible to confirm or reject these hypotheses. The positive cougar of this project was a roadkill animal; thus, post-mortem

contamination of trachea is a possibility, particularly as the Ct values were high (Ct = 34.5). Histopathological examinations, apart from the detection of parasites in the small intestine, did not offer more valuable information due to the advanced state of autolysis of the material. Nevertheless, future studies investigating the pathogenicity of RVA in wild felids are warranted.

FINAL CONSIDERATIONS

7 FINAL CONSIDERATIONS

This is the first molecular screening of AstV, EMCV and RVA in wild felids species from the New World. Furthermore, it characterizes the first molecular detection of AstV and RVA in these species. Although their pathogenicity remains to be investigated, it is important to record that these viruses are circulating in wild Brazilian felids, especially considering that one of the positive animals was a free-ranging individual. Ongoing studies will further characterize the constellation genotype of the detected RVA to confirm whether it is a feline related genotype or a potential novel one.

In addition, a vulnerable species (the southern tiger cat) was positive for a potential novel AstV species. Therefore, continuous monitoring of AstV as well as research on their pathogenicity in these species are important, mostly in animals under human care, since they are susceptible to stress and may develop unknown clinical manifestations.

Finally, although yielding negative results, to our knowledge, EMCV was screened for the first time in Brazilian wild felids. It might be that this virus has a low circulation amongst wild felids in Brazil, but further studies with greater sampling will be necessary to confirm it.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

REFERENCES

8 BIBLIOGRAPHIC REFERENCES

ADANIA, C.H.; SILVA, J.C.; FELIPPE, P.A.N. Capítulo 37: Carnivora – Felidae (Onça, Suçuarana, Jaguatirica e Gato-do-mato). *In:* CUBAS, Z.S.; SILVA, J.C.; CATÃO-DIAS, J.L. **Tratado de animais selvagens: Medicina Veterinária.** 2ªEd. São Paulo: Editora REN/Roca, p.864-906. 2014

ALARCÓN, R.G.D.; LIOTTA, D.J.; MIÑO, S. Zoonotic RVA: State of the Art and Distribution in the Animal World. **Viruses**, 13, 2554. 2022

ALMEIDA, P.R.; LOREZENTTI, E.; CRUZ, R.S.; WATANABE, T.T.; ZLOTOWSKI, P.; ALFIERI, A.A.; DRIEMEIER, D. Diarrhea caused by *Rotavirus A, B* and *C* in suckling piglets from Southern Brazil: molecular detection and histologic and immunohistochemical characterization. **J Vet Diag Investig**, v.30, n.3, p.370-376. 2018

ALVES, C.D.B.T.; BUDASZEWSKI, R.F.; CIBULSKI, S.P.; WEBER, M.N.; MAYER, F.Q.; BIANCHI, M.V.; ZALAFON-SILVA, B.; KONRADT, G.; SLAVIERO, M.; SONNE, L.; DRIEMEIER, D.; ALIEVI, M.M.; CANAL, C.W. *Mamastrovirus* 5 detected in a crab-eating fox (*Cerdocyon thous*): Expanding wildlife host range of astroviruses. **Comp Immunolog Microbiol and Infec Dis**. 58, p.36-43, 2018.

AO, Y.; XU, J.; DUAN, Z. A novel cardiovirus species identified in feces of wild Himalayan marmots. **Infec, Genet & Evol**; 103(105347). 2022

ATKINS, A.; WELLEHAN, JR., J.F.X.; CHILDRESS, A.L.; ARCHIER, L.L.; FRASER, W.A.; CITINO, S.B. Characterization of an outbreak of astroviral diarrhea in a group of cheetahs (*Acinonyx jubatus*). **Vet Microbiol**, 134, p.160-165. 2009

ATTOUI, H.; MERTENS, P.P.C.; BECNEL, J. ; BELAGANAHALLIS, S. ; BERGOIN, M, ; BRUSSARD, C.P. ; CHAPPELL, J.D. ; CIARLET, M. ; DEL VAS, M. ; DERMODY, T.S. ; DORMITZER, P.R. ; DUNCAN, R. ; FANG, Q. ; GRAHAM, R. ; GUGLIELMI, K.M. ; HARDING, R.M. ; HILLMAN, B. ; MAKKAY, A. ; MARZACHÌ, C. ; MATTHIJNSSENS, J. ; MILNE, R.G. ; MOHD J.F. ; MORI, H. ; NOORDELOOS, A.A. ; OMURA, T. ; PATTON, J.T. ; RAO, S. ; MAAN, M. ; STOLTZ, D. ; SUZUKI, N. ; UPADHYAYA, N. M. ; WEI, C. ; ZHOU, H. Family *Reoviridae. In :* Chapter Version : **ICTV Ninth Report**. 2009

BACKUES, K.A.; HILL, M.; PALMENBERG, A.C.; MILLER, C.; SOIKE, K.F.; AGUILAR, R. Genetically Engineered Mengo Virus Vaccionation of Multiple Captive Wildlife Species. **J Wildl Dis**, v.35, n.2, p.384-387. 1999

BILLINIS, C. Encephalomyocardiditis Virus Infection in Wildlife Species in Greece. **J Wildl Dis**, v.45, n.2, p.522-526. 2009

BOROS, A.; ALBERT, M.; PANKOVICS, P.; BÍRÓ, H.; PASAVENTO, P.A.; PHAN, T.G.; DELWART, E.; REUTER, G. Outbreakf of Neuroinvasive Astrovirus Associated

with Encephalomyelitis, Weakness, and Paralysis among Weaned Pigs, Hungary. **Emerg Infec Dis**, vol.23, n.12, p.1999-2010. 2017

BOROS, A.; ORLOVÁCZ, K.; PANKOVICS, P.; SZEKERES, S.; FÖLDVÁRI, G.; FASHBENDER, E.; DELWART, E.; REUTER, G. Diverse picornaviruses are prevalente among free-living and laboratory rats (*Rattus norvegicus*) in Hungary and can cause disseminated infections. **Infec, Genet & Evol.** 75:103988. 2019

BOSCH, A.; GUIX, S.; KRISHNA, N.K.; MÉNDEZ, E.; MONROE, S.S.; PANTIN-JACKWOOD, M.; SCHULTZ-CHERRY, S. Family: *Astroviridae. In:* Chapter Version: **ICTV Ninth Report**. 2009

BRADLEY, C.A.; ALTIZER, S. Urbanization and the ecology of wildlife diseases. **TRENDS in Ecol and Evol**, v.22, n.2. 2006

CAETANO, A.; BARBOSA, G.; DÁBILLA, N.; FRANCO, F.C.; FIACCADORIS, F.S.; SOUZA, M. First report of human astrovirus MLB1 in a child from Mid-West Brazil. **J Med Virol.** 93:3286-3287. 2021

CANELLI, E.; LUPPI, A.; LAVAZZA, A.; LELLI, D. ; SOZZI, E. ; MARTIN, A.M.M. ; GELMETTI, D.; PASCOTTO, E.; SANDRI, C.; MAGNONE, W.; CORDIOLI, P. Encephalomyocarditis virus infection in an Italian zoo. **Virol J**, 7:64. 2010

CARDETI, G.; MARIANO, V.; ELENI, C.; ALOISI, M.; GRIFONI, G.; SITTINIERI, S. ; DANTE, G. ; ANTOGNETTI, V. ; FOGLIA, E.A. ; CERSINI, A.; NARDI, A. Encephalomyocarditis vírus infection in *Macaca sylvanus* and *Hystrix cristata* from na Italian rescue centre for wild and exotic animals. Virol J, 13:193. 2016

CAROCCI, M.; BAKKALI-KASSIMI, L. The encephalomyocarditis virus. **Virulence**, v.3, n.4, p.351-367. 2012

CAUSEY, O.R.; SHOPE, R.E.; LAEMMERT, H. Report of an epizootic of Encephalomyocarditis virus in Pará, Brazilk. **Rev Serv Esp Saúde Públi.** Rio de Janeiro, v. 12, n.1, p.47-50. 1962

CAVALCANTI, S.C.; MARCHINI, S. ; ZIMMERMANN, A. ; GESE, E.M. ; MACDONALD, D.W. Chapter 17: Jaguars, Livestock, and People in Brazil: Realities and Perceptions Behind The Conflict. *In:* MACDONALD, D. & LOVERIDGE, A. **The biology and conservation of wild felids.** 1ªEd. Oxford University Press, Oxford (UK), p.383-402. 2010

CHAE, S.B.; JEONG, C.G.; PARK, J.S.; NA, E.J.; OEM, J.K. Detection and Genetic Characterization of Astroviruses in Brain Tissues of Wild Raccoon Dogs. **Viruses**, 15, 1488. 2023

CRAWFORD, S.E.; PATEL, D.G.; CHENG, E.; BERKOVA, Z.; HYSER, J.M.; IARLET, M.; FINEGOLD, M.J.; CONNER, M.E.; ESTES, M.K. Rotavirus viremia and extraintestinal viral infection in the neonatal rat model. **J Virol**. 80, p.4820-4832. 2006

CZECHOWICZ, J.; HUAMAN, J.L.; FORSHEY, B.M.; MORRISON, A.C.; CASTILLO, R.; HUAMAN, A.; CACEDA, R.; EZA, D.; ROCHA, C.; BLAIR, P. J.; OLSON, J.G.; KOCHEL, T.J. Prevalence and Risk Factors for Encephalomyocarditis Virus Infection in Peru. Vector-borne & Zoonotic Diseases. v.11, n.4, p.367-374. 2011

DE BARROS, B.C.V.; CHAGAS, E.N.; BEZERRA, L.W.; RIBEIRO, L.G.; DUARTE JR., J.W.B.; PEREIRA, D.; PENHA JR., E.T.; SILVA, J.R.; BEZERRA, D.A.M; BANDEIRA, R.S.; PINHEIRO, H.H.C.; GUERRA, S.F.S.; GUIMARÃES, R.J.P.S.; MASCARENHAS, J.D.A.P. *Rotavirus A* in wild and domestic animals from areas with environmental degradation in the Brazilian Amazon. **PLoS ONE**, v.13, n.12:e0209005. 2018

DE GRAZIA, S.; GIAMMANCO, G.M.; POTGIETER, C.A.; MATTHIJNSSENS, J.; BÁNYAI, K.; PLATIA, M.A.; COLOMBA, C.; MARTELLA, V. Unusual Assortment of Segments in 2 Rare Human *Rotavirus* Genomes. **Emerg Infec Dis**, v.16, n.5, p.859-862. 2010

DIAN, Z.; SUN, Y.; ZHANG, G.; XU, Y.; FAN, X.; YANG, X.; PAN, Q.; PEPPELENBOSCH, M.; MIAO, Z. Rotavirus-related systemic diseases: clinical manifestation, evidence and pathogenesis. **Critical Reviews in Microbiol**, v.47, n.5, p.580-595. 2021

DONG, G.; WANG, Q.; NIU, J.; GUP, Y.; ZHAO, H.; ZHANG, S.; WANG, K.; HU, G.; YI, S. Development and application of a TaqMan-based real-time PCR assay for specifically detecting feline astrovirus. **Mol & Cel Probes**, 57:101729. 2021

FENG, R.; WEI, J.; ZHANG, H.; FAN, J.; LI, X.; WANG, D.; XIE, J.; QIAO, Z.; LI, M.; BAI, J.; MA, Z. National serosurvey encephalomyocarditis virus in healthy people and pigs in China. **Arch Virol**, 160:2957-2964. 2015

FERNANDEZ-CASSI, X.; MATÍNEZ-PUCHOL, S.; SILA-SALES, M.; CORNEJO, T.; BARTOLOME, R.; BOFILL-MASS, S.; GIRONES, R. Unveilling Viruses Associated with Gastroenteritis Using a Metagenomics Approach. **Viruses**, 12:1432. 2020

FLACKE, G.L.; TKALCIC, S; STECK, B.; WARREN, K.; MARTIN, G.B. A Retrospective Analysis of Mortality in Captive Pygmy Hippopotamus (*Choeropsis liberiensis*) from 1912 to 2014. **Zoo Biol.** 9999, p.1-14. 2016

FOGLIA, E.A.; PEZZONI, G.; BONILAURI, P.; TORRI, D.; GRAZIOLI, S.; BROCCHI, E. A recent review about encephalomyocarditis virus circulating in compartmentalized animal population in Northern Italy. **Nature**, 13:592. 2023

FOLEY, J.E.; SWIFT, P.; FLEER, K.A.; TORRES, S.; GIRARD, Y.A.; JOHNSON, C.K. Risk factors for exposure to feline pathogens in California mountain lions (*Puma concolor*). **J Wildl Dis**, v.49, n.2, p.279-293. 2013

GABRIEL, A.M.; PEREIRA, W.L.A.; SILVA, K.S.M.; KAIANO, J.H.L.; NOBRE, A.F.S.; MASCARENHAS, J.D.A.P.; LINHARES, A.C. Group A rotavirus gastroenteritis in orphaned *Leopardus tigrinus* and |*Leopardus pardalis* (Carnivora: Felidae) in the Eastern Amazon. **Rev Pan Amaz Saúde**, 14e202301030. 2023

GANGOPADHAYYA, A.; LOLE, K.; GHUGE, O.; RAMDASI, A.; KAMBLE, A.; ROY, D.; THAKAR, S.; NATH, A.; SUDEEP, A.; CHERIAN, S. Metagenomic Analysis of Viromes of *Aedes* Mosquitoes across India. **Viruses**, v.16, n.109. 2024

GERMAN, A.C.; ITURRIZA-GÓMARA, M.; DOVE, W.; SANDRASEGARAM, M.; NAKAGOMI, T.; NAKAGOMI, O.; CUNLIFFE, N.; RADFORD, A.D.; MORGAN, K.L. Molecular Epidemiology of *Rotavirus* in Cats in the United Kingdom. **J Clin Microbiol**, v.53, n.2, p.455-464. 2015

GHOSH, S.; KOBAYASHI, N. Exotic rotaviruses in animals and rotaviruses in exotic animals. **Virus Dis**, v.25, n.2, p.158-172. 2014

GREENE, C.E. Infecções Entéricas Virais em Felinos. *In:* GREENE, C.E. **Doenças Infecciosas em Cães e Gatos.** 4ªEd. Rio de Janeiro: Guanabara Koogan, p.190-215. 2015

GRIS, A.H.; ALVES, R.S.; CAMARGO, L.J.; BAUMBACH, L.F.; MENEGATT, J.C.O.; SILVA, E.M.S.; PEROSA, F.F.; LIMA, R.P.; BERTOLINI, M.; FIÚZA, A.T.L.; WATANABE, T.T.N.; CANAL, C.W.; DRIEMEIER, D. Reemerging of Encephalomyocarditis Virus in Pigs in Brazil: Pathological and Viral Characterization. **Transb & Emerg Dis**, 6582778. 2023

GUIX, S.; BOSCH, A.; PINTÓ, R.M. Chapter 6: *Astrovirus* Taxonomy. *In:* SCHULTZ-CHERRY, S. ***Astrovirus* Research: Essential Ideas, Everyday Impacts, Future Direction**. 1ªEd. Springer Science + Business Media New York, p.97-118. 2013

GUTIÉRREZ, S.; YVON, M.; PIROLLES, E. ; GARZO, E. ; FERERES, A. ; MICHALAKIS, Y.; BLANC, S. Circulating Virus Load Determines the Size of Bottlenecks in Viral Populations Progressing within a Host. **PLoS Pathog**, v.8, n.11, e10030009. 2012

HOQUE, S.A.; KOTAKI, T. ; PHAM, N.T.K.; ONDA, Y.; OKITSU, S.; SATO, S.; YUKI, Y.; KOBAYASHI, T.; MANEEKARN, N.; KLYONO, H.; HAYAKAWA, S.; USHIJIMA, H. Genotype Diversity of Enteric Viruses in Wastewater Amid the COVID-19 Pandemic. **Food & Environ Virol**, v.15, p.176-191. 2023

HUANG, X.; HOU, J.; LE, X. ; HOU, Y. ; YANG, L. ; LI, Q. ; WANG, B. ; XIA, X. Diversity of astroviruses in wild animals in Yunnan province, China. **Virol J**, v.21, n.51, 2024

INSTITUTO CHICO MENDES DE CONSERVAÇÃO DA BIODIVERSIDADE (ICMBio). **Plano de Manejo do Parque Nacional Serra da Capivara**. Brasília, julho de 2019.

INTERNATIONAL UNION FOR CONSERVATION OF NATURE. **The IUCN Red List of Threatened Species.** 2022

JOHANSSON, O.; ULLMAN, K.; LKHAGVAJAV, P.; WISEMAN, M.; MALMSTEN, J.; LEIJON, M. Detection and Genetic Characterization of Viruses Present in Free-Ranging Snow Leopards Using Next-Generation Sequencing. **Front Vet Sci.** 7:645. 2020

KISHIMOTO, M.; HANG'OMBE, B.M.; HALL, W.W.; ORBA, Y.; SAWA, H.; SASAKI, M. *Mastomys natalensis* is a possible natural rodent reservoir for encephalomyocarditis virus. **J Gen Virol.** 102:0015644. 2021

KNOWLES, N.J.; HOVI, T.; HYYPIÄ, T.; KING, A.M.Q.; LINDBERG, A.M.; PALLANSCHI, M.A.; PALMENBERG, A.C.; SIMMONDS, P.; SKERN, T.; STANWAY, G.; YAMASHITA, T.; ZELL, R. Family: *Picornaviridae. In:* Chapter Version: **ICTV Ninth Report**. 2009

KOENEN, F. ; VANDERHALLEN, H.; CASTRYCK, F.; MIRY, C. Epidemiologic, Pathogenic and Molecular Analysis on Recent Encephalomyocarditis Outbreaks in Belgium. **J Vet Med B**, v. 46, p.217-231. 1999

KUCZERA, K.; ORŁOWSKA, A.; SMRECZAK, M.; FRANT, M.; TREBAS, P.; ROLA, J. Prevalence of Astroviruses in Different Animal Species in Poland. **Viruses**, v. 16, n.80. 2024

KUMAR, S.; STECHER, G.; TAMURA, K. MEGA7: Molecular Evolutionary Genetic Analysis Version 7.0 for Bigger Datasets. **Molecular Biology and Evolution**. v.33, p.1870-1874. 2016

KUMTHIPS, K.; KHAMRIN, P.; USHIJIMA, H.; MANEEKARN, N. Detection of Six Different Human Enteric Viruses Contaminating Environmental Water in Chiang Mai, Thailand. **Micrbiol Spectrum**, v.11, n.1. 2023

LABARGE, L.; EVANS, M.J.; MILLER, J.R.B.; CANNATARO, G.; HUNT, C.; ELBROCH, L.M. Pumas (*Puma concolor*) as ecological brokers: a review of their biotic relationships. **Mammal Review**, p.360-376, 2021

LAWLER, P.E.; COOK, A.K.; WILLIAMS, H.G.; ARCHER, L.L.; SCHAEDEL, K.E.; ISAZA, N.M.; WELLEHAN JR., J.F.X. Determination of the diversity of astroviruses in feces from cats in Florida. **J Vet Diag Invest**, v.20, n.2, p.275-279. 2018

LESTARI, F.B. ; CHANDRANOL, K. ; CHUCHAONA, W. ; VONGPUNSAWAD, S. ; POOVORAWAN, Y. A G3P[9] *Rotavirus* strain with an unusual genome constellation in a diarrheic cat in Thailand. **Arch Virol**, 168:24. 2023

LI, Y.; GORDON, E.; IDLE, A.; HUI, A.; CHAN, R. ; SEGUIN, M.A. ; DELWART, E. Astrovirus Outbreak in an Animal Shelter Associated with Feline Vomiting. **Front Vet Sci**, 8:628082. 2021

LI, Y.; MIYANI, B.; FAUST, R.A. ; DAVID, R.E. ; XAGORAKAI, I. A broad wastewater screening and clinical data surveillance for virus-related disease in the metropolitan Detroit area in Michigan. **Hu Gen**, v.18, n.14. 2024

LIPTON, H.L.; KUMAR, A.S.M.; HERTZLER, S. Chapter 12: Cardioviruses: Encephalomyocarditis Virus and Theiler's Murine Encephalomyelitis Virus. *In:* The **Mouse in Biomedical Research**. 2ªEd, p.311-324. 2007

LIU, H.; YAN, Q.; ZHAO, B.; LUO, J.; WANG, C.; D, Y.; YAN, J.; HE, H. Isolation, molecular characterization, and phylogenetic analysis of encephalomyocarditis virus from South China tigers in China. **Infec Gen & Evol**, v.19, p.240-243, 2013.

LU, L.; ASHWORTH, J.; NGUYEN, D.; LI, K.; SMITH, D.B.; WOOLHOUSE, M. No Exchange of Picornaviruses in Vietnam Between Humans and Animals in a High-Risk Cohort with Close Contact Despite High Prevalence and Diversity. **Viruses**, v.13. 2021

LUM, S.H.; TURNER, A.; GUIVER, M.; BONNEY, D.; MARTLAND, T.; DAVIES, E.; NEWBOULD, M.; BROWN, J.; MORFOPOULOUS, S.; WYNN, R. An emerging opportunistic infection: fatal *astrovirus* (VA1/HMO-C) encephalitis in a pediatric stem cell transplant recipient. **Transpl Intect Dis**, v.18, n.6, p.960-964. 2016

LYNCH, M.; SHIEH, W.J.; TATTI, K.; GENTSCH, J.R.; HARRIS, T.F.; JIANG, B.; GUARNER, J.; BRESEE, J.S.; GREENWALD, M.; CULLEN, S. et al. The pathology of rotavirus-associated deaths, using new molecular diagnostics. **Clin Infect Dis**. 37, p.1327-1333. 2003

MADI, N.; AL-NAKIB, W.; MUSTAFA, A.S.; HABIBI, N. Metagenomic analysis of viral diversity in respiratory samples from patients with respiratory tract infections in Kuwait. **J Med Virol**. 90, p.410-420. 2018

MAESTRI, R.P.; KAIANO, J.H.L.; NERI, D.L.; SOARES, L.S.; GUERRA, S.F.S.; OLIVEIRA, D.S.; FARIAS, Y.N.; GABBAY, Y.B.; LEITE, J.P.G.; LINHARES, A.C.; MASCARENHAS, J.D.A.P. Phylogenetic Analysis of Probable Non-Human Genes of Group A Rotaviruses Isolated from Children with Acute Gastroenteritis in Belém, Brazil. **J Med Virol**, v.82, p.1993-2002. 2012

MARSHALL, J.A.; KENNETT, M.L.; RODGER, S.M.; STUDDERT, M.J.; THOMPSON, W.L.; GUST, I.D. Virus and virus-like particles in the faeces of cats with and without diarrhoea. **Australian Vet J**, v. 64, n.4, p.100-105. 1987

MARTELLA, V.; POTGIETER, A.C.; LORUSSO, E. ; DR GRAZIA, S.; GIAMMANCO, G.M.; MATTHIJNSSENS, J.; BÁNYAI, K.; CIARLET, M.; LAVAZZA, A.; DECARO, N.; BUONAVOCLIA, C. A feline rotavirus G3P[9] carries traces of multiple reassortment events and resembles rare human G3P[9] rotaviruses. **J Gen Virol**, v. 92, p.1214-1221. 2011

MCLELLAND, D.J.; KIRKLAND, P.D.; KARRIE, A.R.; DIXON, R.J.; SMITH, N. Serologic responses of barbary sheep (*Ammotragus lervia*), Indian antelope (*Antilope cervicapra*), wallaroos (*Macropus robustus*), and chimpanzees (*Pan troglodytes*) to an inactivated encephalomyocarditis virus vaccine. **J Zoo Wildl Med**, v. 36, n.1, p. 69-73. 2005

MEDKOUR, H.; CASTANEDA, S.; AMONA, I.; FENOLLAR, F.; ANDRÉ, C.; BELAIS, R.; MUNGONGO, P.; MUYEMBÉ-TAMFUM, J.J; LEVASSEUR, A.; RAOULT, D.; DAVOUST, B.; MEDIANNIKOV, O. Potential zoonotic pathogens hosted by endangered bonobos. **Nature**, 11:6331. 2021

MINISTÉRIO DO MEIO AMBIENTE, DOS RECURSOS HÍDRICOS E DA AMAZÔNIA LEGAL. **Plano de Manejo – Reserva Biológica do Gurupi.** 1999

MIURA, T.; KADOYA, S.; TAKINO, H.; SANO, D.; AKIBA, M. Temporal variations of human and animal *Rotavirus A* genotypes in surface water used for drinking water production. **Front Microbiol**, 13:912147. 2022

NAKAGOMI, T.; NAKAGOMI, O. RNA-RNA Hybridization Identifies a Human Rotavirus that is Genetically Related to Feline Rotavirus. **J Virol**, v. 63, n. 3, p.1431-1434. 1989

NG, T.F.F.; MESQUITA, J.R.; NASCIMENTO, M.S.J.; KONDOV, N.O.; WONG, W.; REUTER, G.; KNOWLES, N.J.; VEJA, E.; ESONA, M.D.; DENG, X.; VINJÉ, J.; DELWART, E. Feline fecal virome reveals novel and prevalente enteric viroses. **Vet Microbiol**, v. 171, p.102-111. 2014

NGUYEN, T.V.; PIEWBANG, C.; TECHANGAMSUWAN, S. Genetic characterization of canine astrovirus in non-diarrhea dogs and diarrhea dogs in Vietnam and Thailand reveals the presence of a unique lineage. **Front Vet Sci**, 10:1278417. 2023

OBERSTE, M.S.; GOTUZZO, E.; BLAIR, P.; NIX, W.A.; KSIAZEK, T.G.; COMER, J.A.; ROLLIN, P.; GOLDSMITH, C.S.; OLSON, J.; KOCHEL, T.J. Human Febrile Illness Caused by Encephalomyocarditis Virus Infection, Peru. **Emerg Infec Dis**, v. 15, n. 4, p.640-646. 2009

O’CONNOR, T.W.; FINLAISON, D.S. ; MANNING, L.K. ; HAZELTON, M.S.; SPIERS, Z.B.; PINCZOWSKI, P.; BOLIN, E.M.; KEMSLEY, P.D.; HORADAGODA, N.U.; DAR, A.J.; COOK, R.W.; KIRKLAND, P.D. Encephalomyocarditis virus infection in alpacas. **Australian Vet J**. 2020

OLAIMAT, A.N.; TAYBEH, A.O.; AL-NABULSI, A.; AL-HOLY, M.; HATMAL, M.M.; ALZYOUD, J; AOLYMAT, I.; ABUGHOUSH, M.H.; SHAHBAZ, H.; ALYZOUD, A.; OSAILI, T.; AYYASH, M.; COOMBS, K.M.; HOLLEY, R. Common and Potential Emerging Foodborne Viruses: A Comprehensive Review. **Life**, v.14, n.190. 2023

OLIVARES, A.I.O.; MORAES, M.T.B.; QUEIROZ, H.C.F.; PIMENTA, Y.C.; LEITE, J.P.G. First report of human astrovirus MLB2 in Brazil detected in feces of children with acute gastroenteritis living in the state of Roraima, Northern Brazil. **Braz J Infect Dis.** 2020

PACINI, M.I.; MAZZEI, M.; SGORBINI, M.; D’ALFONSO, R.; PAPINI, R.A.A. One-Year Retrospective Analysis of Viral and Parasitological Agents in Wildlife Animals Admitted to a First Aid Hospital. **Animals**, v.13, n.931. 2023

PÉROT, P.; LECUIT, M.; ELOIT, M. Astrovirus Diagnostics. **Viruses**, v.9, n.10. 2017

PHAN, T.G.; LEUTENEGGER, C.M.; CHAN, R.; DELWART, E. *Rotavirus I* in feces of a cat with diarrhea. **Virus Genes**, v.53, p.487-490. 2017

QURESHI, M.I.; WORTHINGTON, B.M.; LIU, Y.; CHEUNG, W.Y.M.; SU, S.; ZHENG, Z.; LI, L.; LAM, T.T.Y.; GUAN, Y.; ZHU, H. Discovery of novel Mamastroviruses in Bactrian camels and dromedaries reveals complex recombination history. **Virus Evol**, v.9, n.1, p.1-14. 2023

RAO, G.; CAPONE, D.; ZHU, K.; KNOBLE, A.; LINDE, Y.; CLARK, K.; LAI, A.; KIM, J.; HUANG, C.; BIVINS, A.; BRONW, J. Simultaneous detection and quantification of multiple pathogens targets in wastewater. **PLoS NTDs.** 2023

REDDACLIFF, L.A.; KIRKLAND, P.D.; HARTLEY, W.J.; REECE, R.L. Encephalomyocarditis Virus Infections in an Australian Zoo. **J Zoo Wildl Med**, v.28, n.2, p.153-157. 1997

RIVERA, R.; NOLLENS, H.H.; VENN-WATSON, S.; GULLAND, F.M.D.; WELLEHAN JR., J.F.X. Characterization of phylogenetically diverse astroviruses of marine mammals. **J Gen Virol**, v.91, p.166-173. 2009

ROMEY, A.; LAMGLAIT, B.; BLANCHARD, Y. ; TOUZANI, F. ; QUENAULT, H. ; RELMY, A. ; ZIENTARA, S. ; BLAISE-BOISSEAU, S. ; BAKKALI-KASSIMI, L. Molecular characterization of encephalomyocarditis virus strains isolated from an African Elephant and rats in a French zoo. **J Vet Diag Investig**, v.33, n.2, p.313-321. 2021

SANTOS, N.; VOLOTÃO, E.M.; SOARES, C.C.; ALBUQUERQUE, M.C.M.; SILVA, F.M.; CARVALHO, T.R.B.; PEREIRA, C.F.A.; CHIZIKOV, V.; HOSHINO, Y. *Rotavirus* Strains Bearing Genotype G9 or P[9] Recovered from Brazilian Children with Diarrhea from 1997-1999. **J Clin Microbiol**, v. 39, n.3, p.1157-1160. 2001

SANTOS, R.A.T.; CARDOSO, D.D.P. Astrovírus. **Rev Patol Trop**, v.34, n.3, p.161-174. 2005

SATTER, S.M.; KATZ, E.; HOSSAIN, M.E.; FARIHA, F.; TALHA, M.; SMART, S.L.; BOWEN, M.D.; RAHMAN, M.; PARASHAR, U.D.; CORTESE, M.M. Detection of Rotavirus in Respiratory Specimens From Bangladeshi Children Aged < 2 Years Hospitalized for Acute Gastroenteritis. **J of Infec Dis**. 229, p.457-461. 2024

SEWARD, N.W.; VERCAUTEREN, K.C.; WITMER, G.W.; ENGEMAN, R.M. Feral Swine Impacts on Agriculture and the Environment. **Sheep & Goat Res J**, v.19, n.12, p.34-40. 2004

SMITH, K.F.; ACEVEDO-WHITEHOUSE, K.; PEDERSEN, A.B. The role of infectious diseases in biological conservation. **Animal Conservation**, v.12, p.1-12. 2009

SOMA, T.; OGATA, M.; OHTA, K.; YAMASHITA, R.; SASAI, K. Prevalence of *Astrovirus* and *Parvovirus* in Japanese domestic cats. **J Vet Med Sci**, v.82, n.9, p.1243-1246. 2020

SQUIRES, R.A. An update on aspects of viral gastrointestinal diseases of dogs and cats. **New Zeal Vet J**, v.51, n.6, p.252-261. 2003

STOCKDALE, S.R.; BLANCHARD, A.M.; NAYAK, A.; HUSAIN, A.; NASHINE, R.; DUDANI, H.; MCCLURE, P.C.; TARR, A.W.; NAG, A.; MEENA, E,; SINHA, V.; SHRIVASTAVA, S.K.; HILL, C.; SINGER, A.C.; GOMES, R.L.; ACHEAMPONG, E.; CHIDAMBARAM, S.B.; BHATNAGAR, T.; VETRIVEL, U,; ARORA, S.; KASHYAP, R.S.; MONAGHAN, T.M. RNA-Seq of untreated wastewater to assess COVID-19 and emerging and endemic viruses for public health surveillance. **The Lancet Regional Health – Southeast Asia**, 14:100205. 2023

SUN, N.; YANG, Y.; WANG, G.S.; SHAO, X.Q.; ZHANG, S.Q.; WANG, F.X.; TAN, B.; TIAN, F.L.; CHENG, S.P.; WEN, Y.J. Detection and Characterization of Avastrovirus Associated with Diarrhea Isolated from Minks in China. **Food Environ Virol.** Springer Sciene+Business Media New York. 2014

TABOADA, B.; ESPINOZA, M.A.; ISA, P.; APONTE, F.W.; ARIAS-ORTIZ, M.A.; MONGE-MARTÍNEZ, J.; RODRÍGUEZ-VÁZQUEZ, R.; DÍAS-HERNÁNDEZ, F.; ZÁRATE-VIDAL, F.; WONG-CHEW, R.M. et al. Is there still room for novel viral pathogens in pediatric respiratory tract infections? **PLoS One**. 9:e113570. 2014

THI KHA TU, N.; THI THU HONG, N. ; THI HAN NY, N.; MY PHUC T.; THI THANH TAM, P.; DOOM, H.R.; DANG TRUNG NGHIA, H.; THAO HUONG, D.; AN HAN, D.; THI THU HA, L. et al. The virome of acute respiratory diseases in individuals at risk of zoonotic infections. **Viruses**. 12:960. 2020

TRUONG, Q.L.; SEO, T.W.; YOON, B.I.; KIM, H.C.; HAN, J.H.; HAHN, T.W. Prevalence of Swine Viral and Bacterial Pathogens in Rodents and Stray Cats Captured around Pig Farms in Korea. **J Vet Med Sci**. v.75, n.12, p.1647-1650. 2013

VAN BRUSSEL, K.; WANG, X.; SHI, M.; CARRAI, M.; LI, J. ; MARTELLA, V. ; BEATTY, J.A. ; HOLMES, E.C. ; BARRS, V. R. Identification of Novel Astroviruses in the Gastrointestinal Tract of Domestic Cats. **Viruses**, v.12, 1301. 2020

VANDERHALLEN, H.; KOENEN, F. Identification of Encephalomyocarditis Virus in Clinical Samples by Reverse Transcription-PCR Followed by Genetic Typing Using Sequence Analysis. **J Clin Microbiol**, v.36, n.12, p.3463-3467. 1998

VERCAMMEN, F.; BOSSELER, L.; TIGNON, M. ; CAY, A.B. Encephalomyocarditis virus in a captive Malayan tapir (*Tapirus indicus*). **Open Vet J**, v.7, n.2, p.100-103

VYSHEMIRSKII, O.I.; AGUMAVA, A.A.; KALAYDZYAN, A.A.; LEONTYUK, A.V.; KUHN, J.H.; SHCHETININ, A.M.; VISHNEVSKAYA, T.V.; EREMYAN, A.A.; ALKHOVSKY, S.V. Isolation and genetic characterization of encephalomyocarditis virus 1 from a deceased captive hamadryas baboon. **Virus Res**, v. 244, p.164-172. 2018

WANG, Y.; GUO, X.; COL, Y.; ZHOU, Y.; YANG, K.; FU, Z.; SUN, J.; LIU, G.; CHENG, B.; JIANG, S.; LI, Y. Genetic characterization and phylogenetic analysis of feline astrovirus from Anhui province in Eastern China. **3 Biotech**, v.10:354. 2020

WANG, Y.; FU, Z.; GUO, X.;, ZHANG, D.; BAI, C.; LI, W.; LIU, G.; LI, Y. ; JIANG, S. Development of SYBR Green I-based real-time reverse transcription polymerase chain reaction for the detection of feline astrovirus. **J Virol Methodol**, 288:114012. 2021

WERID, G.M.; IBRAHIM, Y.M.; CHEN, H.; FU, L.; WANG, Y. Molecular Detection and Genetic Characterization of Potential Zoonotic Swine Enteric Viruses in Northern China. **Pathogens**, 11:417. 2022a

WERID, G.M.; ZHANG, H.; IBRAHIM, Y.M.; PAN, Y.; ZHANG, L.; XU, Y.; ZHANG, W.; WANG, W.; CHEN, H.; FU, L.; WANG, Y. Development of a Multiplex RT-PCR Assay for Simultaneous Detection of Four Potential Zoonotic Swine RNA Viruses. **Vet Sci**, 9:176. 2022b

WILDI, N.; SEUBERLICH, T. Neurotropic Astroviruses in Animals. **Viruses**, 13:1201. 2021

YI, S.; VIU, J.; WANG, H.; DONG, G.; GUO, Y.; DONG, H.; WANG, K.; HU, G. Molecular characterization of feline astrovirus in domestic cats from Northeast China. **PLoS ONE**, v.13, n.1, e0205441. 2018

YUAN, W.; ZHENG, Y.; SUN, M.; ZHANG, X.; QI, Y.; SUN, J. Development of a TaqMan-based real-time reverse transcription polymerase chain reaction assay for the detection of encephalomyocarditis virus. **J Virol Methods**, v. 207, p.60-65. 2014

ZAMORA-FIGUEROA, A.; ROSALES, R.E.; FERNÁNDEZ, R.; RAMÍREZ, V.; BASTARDO, M.; FARÍAS, A.; VIZZIE, E. Detection and diversity of gastrointestinal viruses in wastewater from Caracas, Venezuela, 2021-2022. **Virology**, 589:109913. 2024

ZENG, S.Q.; HALKOSALO, A.; SALMINEN, M.; SZAKAL, E.D.; PUUSTINEN, L.; VESIKARI, T. One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. **J of Virol Methods**, v.153, p.238-240. 2008

ZHANG, H.H.; QIU, Q.G.; LIU, S.J.; HU, W.Q.; LI, J.Y.; LIU, T.N.; PENG, Y.S.; GE, X.Y.; XIAO, C.T. Genomic characterization of a novel astrovirus identified in Amur tigers from a zoo in China. **Arch Virol**, v. 164, p.3151-3155. 2019

ZHAORI, G.T.; FU, L.T.; XU, Y.H.; PENG, Z.J.; SHAN, W.S. Detection of rotavirus antigen in tracheal aspirates of infants and children with pneumonia. **Chin Med J**. 104, p.830-833. 1991

ZHENG, B.J.; CHANG, R.X.; MA, G.Z. ; XIE, J.M.; LIU, Q.; LIANG, X.R.; NG, M.H. Rotavirus infection of the oropharynx and respiratory tract in young children. **J Med Virol**. 34, p.29-37. 1991

ZHUANG, Z.; QIAN, L.; LU, J. ; ZHANG, X. ; MAHMOOD, A.; CUI, L.; WANG, H.; WANG, X.; YANG, S.; JI, L.; SHAN, T.; SHEN, Q.; ZHANG, W. Comparison of viral communities in the blood, feces and various tissues of wild brown rats (*Rattus norvegicus*). **Heliyon**, v.9, e17222. 2023

APPENDIX 1

ID	SPECIES	AGE	GENDER	ORIGIN	SAMPLES
MC4890	*Puma concolor*	Adult	M	Salto de Pirapora (SP)	Blood
MC9440	*Leopardus pardalis*	Adult	M	Zoo	Blood, swab (oral, nasal, rectal)
MC3794	*Leopardus wiedii*	Adult	F	Zoo	Blood, swab (oral, nasal, rectal, vaginal)
MC4769	*Leopardus pardalis*	Adult	F	Zoo	Blood, swab (oral, nasal, rectal, vaginal)
MC4566	*Leopardus guttulus*	Adult	F	Araçoiaba da Serra (SP)	Blood, swab (oral, rectal, vaginal)
MC9574	*Puma concolor*	Adult	M	Zoo	Blood, swab (oral, nasal, rectal)
MC2557	*Leopardus wiedii*	Adult	M	Zoo	Blood, swab (oral, nasal, rectal)
MC3910	*Leopardus wiedii*	Adult	M	Zoo	Blood
MC8105	*Puma concolor*	Young	F	Zoo	Blood, swab (oral, nasal, rectal, vaginal)
RN272.823	*Leopardus guttulus*	Cub	F	Botucatu (SP)	Swab (oral, rectal, vaginal)
bPon543	*Panthera onca*	Adult	M	PNSC (PI)	Oral swab
VIT	*Panthera onca*	Adult	F	Zoo	Blood, swab (oral, nasal, rectal, vaginal)
MC3490	*Herpailurus yagouaroundi*	Adult	F	Zoo	Blood, swab (oral, nasal, rectal, vaginal)
BAN	*Puma concolor*	Young	F	Botucatu (SP)	Blood, swab (oral, nasal, rectal, vaginal)
MEI	*Puma concolor*	Young	F	ReBio Gurupi (MA)	Blood, swab (oral, nasal, rectal, vaginal)
bPon547	*Panthera onca*	Adult	M	ReBio Gurupi (MA)	Rectal swab
CS-001	*Leopardus pardalis*	Adult	M	Caraguatatuba (SP)	Nasal swab, brain, heart, lung, liver, spleen, submandibular lymph node, kidney, intestine, feces, skeletal muscle, tonsils
CS-002	*Leopardus pardalis*	Young	F	Paraibuna (SP)	Nasal swab, brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle, tonsils

CS-009	*Puma concolor*	Young	M	Cajati (SP)	Nasal swab, brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle, tonsils
CS-012	*Leopardus guttulus*	Young	F	Caraguatatuba (SP)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle
CS-014	*Herpailurus yagouaroundi*	Adult	M	Paraibuna (SP)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle
CS-016	*Leopardus guttulus*	Adult	M	Caraguatatuba (SP)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle
CS-018	*Puma concolor*	Adult	F	Corumbataí (SP)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle, tonsils
CS-019	*Leopardus pardalis*	Adult	M	Caraguatatuba (SP)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle, tonsils
CS-025	*Leopardus pardalis*	Adult	M	Duque de Caxias (RJ)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle, tonsils
CS-026	*Leopardus pardalis*	Young	M	Duque de Caxias (RJ)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle, tonsils
CS-027	*Leopardus pardalis*	Adult	F	Duque de Caxias (RJ)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle, tonsils
CS-031	*Puma concolor*	Adult	F	São Miguel do Arcanjo (SP)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine, feces, skeletal muscle, tonsils
RK-032	*Leopardus pardalis*	Young	M	Paraibuna (SP)	Heart, liver, spleen, mesenteric lymph node, kidney, intestine, feces

RK-034	*Puma concolor*	Adult	M	Caraguatatuba (SP)	Brain, heart, lung, spleen, mesenteric lymph node, kidney, intestine
RK-039	*Leopardus guttulus*	Young	F	Caraguatatuba (SP)	Heart, lung, spleen, mesenteric lymph node, kidney, intestine
RK-042	*Puma concolor*	Young	F	Piracicaba (SP)	Heart, lung, intestine
RK-062	*Herpailurus yagouaroundi*	Young	M	Jambeiro (SP)	Brain, heart, liver, mesenteric lymph node
RK-066	*Leopardus pardalis*	Adult	M	Caraguatatuba (SP)	Brain, lung, liver, mesenteric lymph node, kidney
RK-067	*Leopardus pardalis*	Young	M	Miracatu (SP)	Brain, heart
RK-088	*Herpailurus yagouaroundi*	Adult	M	Atibaia (SP)	Heart, lung, liver, spleen, and kidney
RK-089	*Herpailurus yagouaroundi*	Young	M	Atibaia (SP)	Heart, lung, liver, spleen, kidney
RK-104	*Herpailurus yagouaroundi*	Adult	M	Atibaia (SP)	Heart
RK-175	*Leopardus pardalis*	Adult	F	Caraguatatuba (SP)	Brain, heart, lung, liver, kidney,
RK-205	*Herpailurus yagouaroundi*	Adult	M	Paraibuna (SP)	Brain, heart, lung, liver, spleen, kidney
RK-206	*Puma concolor*	Young	F	Registro (SP)	Brain, lung, liver, mesenteric lymph node, kidney
RK-263	*Leopardus pardalis*	Young	M	Ipaussu (SP)	Brain, heart, lung, liver, mesenteric lymph node, kidney
RK-273	*Leopardus pardalis*	Young	F	Barra do Turvo (SP)	Brain, heart, lung, mesenteric lymph node, kidney, intestine
RK-276	*Leopardus pardalis*	Adult	M	Cajati (SP)	Brain, lung, intestine
RK-278	*Leopardus pardalis*	Adult	M	Barra do Turvo (SP)	Brain, heart, lung, liver, spleen, mesenteric lymph node, kidney, intestine
RK-280	*Puma concolor*	Young	M	Cajati (SP)	Brain, lung, spleen, intestine
RK-303	*Leopardus guttulus*	Young	M	Paraibuna (SP)	Brain, heart, liver, mesenteric lymph node, kidney, intestine
RK-310	*Leopardus pardalis*	Adult	M	Caraguatatuba (SP)	Liver

RK-400	*Leopardus pardalis*	Adult	M	Paraibuna (SP)	Brain, heart, lung, liver, mesenteric lymph node, kidney, intestine
RK-414	*Leopardus pardalis*	Adult	M	Paraibuna (SP)	Heart, kidney, intestine, skeletal muscle

ATTACHMENT 1

ATESTADO

Atesto que o Projeto "PESQUISA E CARACTERIZAÇÃO DE AGENTES VIRAIS SELECIONADOS EM FELÍDEOS NÃO DOMÉSTICOS" **Protocolo CEUA 0239/2022** , a ser conduzido por Michelle Colpani, responsável/orientador Prof. José Luiz Catão-Dias, para fins de pesquisa científica/ensino - encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal - CONCEA.

Finalidade	PESQUISA CIENTÍFICA
Vigência do projeto	01/11/2022 a 31/12/2023
Nome Comum / Espécie / Linhagem	FELINA / PANTHERA ONCA / N/A
Raça	N/A
Nº de animais machos	10
Nº de animais fêmeas	10
Nº de animais sexo indefinido	0
Peso médio de animais machos	80,0kg
Peso médio de animais fêmeas	50,0kg
Peso médio de animais sexo indefinido	0
Idade	1 ano(s) e 0 mes(es) e 0 dia(s).
Procedência	Instituições ainda a serem contactadas.

Projeto de Pesquisa aprovado em reunião da CEUA em 07/11/2022

JULIANY GOMES QUITZAN
Presidente da CEUA da FMVZ, UNESP - Campus de Botucatu

Faculdade de Medicina Veterinária e Zootecnia
Seção Técnica Acadêmica
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ATTACHMENT 2

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 84350-2	Data da Emissão: 17/11/2022 08:45:22	Data da Revalidação*: 24/10/2023
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

SISBIO

Nome: MICHELLE COLPANI FERNANDES	CPF: 236.346.528-88
Título do Projeto: PESQUISA E CARACTERIZAÇÃO DE AGENTES VIRAIS SELECIONADOS EM FELÍDEOS NÃO DOMÉSTICOS	
Nome da Instituição: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO	CNPJ: 48.031.918/0020-97

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Coleta e transporte de materiais biológicos de felídeos	12/2022	12/2024

Observações e ressalvas

1	A autorização não eximirá o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VI) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	Deve-se observar as as recomendações de prevenção contra a COVID-19 das autoridades sanitárias locais e das Unidades de Conservação a serem acessadas.
3	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros).
4	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros)
5	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
6	Este documento somente poderá ser utilizado para os fins previstos na Portaria ICMBio nº 748/2022, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
7	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.
8	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
9	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
10	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.

Este documento foi expedido com base na Instrução Normativa nº Portaria ICMBio nº 748/2022. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

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SISBIO

Dados do titular

Nome: MICHELLE COLPANI FERNANDES	CPF: 236.346.528-88
Título do Projeto: PESQUISA E CARACTERIZAÇÃO DE AGENTES VIRAIS SELECIONADOS EM FELÍDEOS NÃO DOMÉSTICOS	
Nome da Instituição: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO	CNPJ: 48.031.918/0020-97

Observações e ressalvas

11	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.

Outras ressalvas

1		CENAP Atibaia-SP
2	Como ressalva informamos: 1. Que o pesquisador titular faça um contato prévio e deve enviar previamente o cronograma de atividades previstas na área via e-mail oficial, evitando todas as possíveis interferências mútuas; 2. Uso de estruturas caso a UC possua: mensagem para gestão da UC com 15 dias antecedência. Informar nº autorização, total pessoas (todas devem estar cadastradas na autorização SISBIO), datas (de chegada e saída), necessidade ou não de uso de alojamento, laboratório, salas etc; 3. Atividade em campo: os locais de coleta devem ser informados para a gestão da UC de forma que não se sobreponham com outras pesquisas em andamento no território; 4. As coletas estão restritas somente ao autorizado, incluindo número de indivíduos coletados, e seguindo o Plano de Manejo de cada UC; 5. O responsável deverá portar cópia desta autorização e documento de identificação pessoal; 6. Marcações/ Sinalizações em Campo: os equipamentos e estruturas que porventura forem instalados em campo devem ser, de preferência, discretas e deverão estar identificados com os dados do projeto e retirados ao término das atividades; 7. Pessoas e atividades estranhas (ex: pescadores, caçadores, visitantes fora da área adequada etc.): devem ser reportadas imediatamente à gestão da UC. 8. Após a conclusão do projeto, o pesquisador deve enviar os resultados (publicações ou qualquer outro material resultante desta pesquisa) para compor o acervo desta Unidade de Conservação; 9. Registros de espécies de interessa da conservação localizados em áreas de visitação devem ser informados à gestão da UC, tão logo seja possível, de forma a privilegiar sua proteção; 10. Programar com os gestores a uma forma de apresentação dos resultados desta pesquisa nas comunidades próximas da área de coleta e para o conselho gestor das UCs." 11. Sempre manter uma boa conduta e ética com os envolvidos no estudo. 12. Em função da pandemia de COVID 19, recomendamos contato com a gestão da UC, para informações referentes ao acesso ao local de estudo, decretos em vigor na presente data e demais orientações respeitando e adotando todos os protocolos de biossegurança nas atividades de campo. 13. Importante o pesquisador apresentar para a gestão da UC um protocolo de segurança a ser adotado em campo; 14. Para uso do Drone, o operador deve vir ao ICMBio assinar o termo de responsabilidade de uso do aparelho e trazer as autorizações e cadastros de voo junto a ANAC e outros órgãos reguladores.	GR2 Nordeste

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Parque Zoológico Municipal Quinzinho de Barros	Sorocaba-SP	Mata Atlântica	Não	Fora de UC Federal
2	Parque Nacional da Serra da Capivara	PI	Caatinga	Não	Dentro de UC Federal
3	CEMPAS - UNESP Botucatu	Botucatu-SP	Cerrado	Não	Fora de UC Federal
4	LAPCOM - Universidade de São Paulo	São Paulo-SP	Mata Atlântica	Não	Fora de UC Federal

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Dados do titular

SISBIO

Nome: MICHELLE COLPANI FERNANDES	CPF: 236.346.528-88
Título do Projeto: PESQUISA E CARACTERIZAÇÃO DE AGENTES VIRAIS SELECIONADOS EM FELÍDEOS NÃO DOMÉSTICOS	
Nome da Instituição: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO	CNPJ: 48.031.918/0020-97

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
5	Mata Ciliar	Jundiaí-SP	Mata Atlântica	Não	Fora de UC Federal

Atividades

#	Atividade	Grupo de Atividade
1	Coleta/transporte de amostras biológicas in situ	Fora de UC Federal
2	Coleta/transporte de amostras biológicas ex situ	Atividades ex-situ (fora da natureza)

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Coleta/transporte de amostras biológicas in situ	Felidae	-
2	Coleta/transporte de amostras biológicas ex situ	Felidae	-

A quantidade prevista só é obrigatória para atividades do tipo "Coleta/transporte de espécimes da fauna silvestre in situ". Essa quantidade abrange uma porção territorial mínima, que pode ser uma Unidade de Conservação Federal ou um Município.

A quantidade significa: por espécie X localidade X ano.

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Carnívoros)	Animal encontrado morto ou partes (carcaça)/osso/pele, Fezes, Fragmento de tecido/órgão, Sangue, Regurgitação/conteúdo estomacal, Outras amostras biológicas(swab nasal, oral e vaginal)

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	UNIVERSIDADE DE SAO PAULO	Laboratório

Este documento foi expedido com base na Instrução Normativa nº Portaria ICMBio nº 748/2022. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

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Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 84350-2	Data da Emissão: 17/11/2022 08:45:22	Data da Revalidação*: 24/10/2023
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

SISBIO

Nome: MICHELLE COLPANI FERNANDES	CPF: 236.346.528-88
Título do Projeto: PESQUISA E CARACTERIZAÇÃO DE AGENTES VIRAIS SELECIONADOS EM FELÍDEOS NÃO DOMÉSTICOS	
Nome da Instituição: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO	CNPJ: 48.031.918/0020-97

Registro de coleta imprevista de material biológico

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

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

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