



Detection of noroviruses in free-ranging jaguars (*Panthera onca*) in the Pantanal, Mato Grosso, Brazil

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Abstract

Nine free-ranging jaguars (*Panthera onca*) were captured, and rectal swabs were collected in the Pantanal of Cáceres, Mato Grosso, Brazil. Reverse transcription polymerase chain reaction specific for noroviruses was performed. Six jaguars (66.6%) tested positive for norovirus genotype GII.11.

Introduction

The genus *Norovirus* of the family *Caliciviridae* includes six genogroups (GI–GVI), and these viruses are the main cause of non-bacterial acute gastroenteritis globally. Their hosts include humans and animals such as pigs, cattle, sheep, sea lions, dogs, domestic cats, rodents, and bats [1]. The detection of diseases in wild animals suggests a risk to endangered species due to potential exposure to infectious agents. Mammals could become disseminators of these agents or could serve as a reservoir for modification of the host-pathogen relationship [2–4]. The aim of this study was to investigate whether noroviruses (NoV) were present in free-living jaguars in a protected area of the Pantanal, Mato Grosso, Brazil.

Materials and methods

Nine jaguars (4 males and 5 females) between the ages of 4 and 8 years were captured during September and December of 2014 at the Taiamã Ecological Station (16°50′34.31″S, 57°35′03.70″W) located in the Pantanal region, approximately 170 km from the city of Cáceres and near the Paraguay River in Mato Grosso, Brazil. Catches were authorized by the National Biodiversity Research System (SISBIO) under licensing number 30896-1. After clinical examination, rectal swabs were collected from all animals. All of the jaguars were apparently healthy, in good physical condition, and had no diarrhea at the time of capture. After all procedures, each animal was released at the same site where it was captured.

RNA was extracted using the protocol of Sokolovski et al. [5]. For reverse transcription polymerase chain reaction (RT-PCR), the forward primer SwNV₁ (5′-CGTACCAGAGGTCAACAAT-3′) and reverse primer SwNV₂ (5′-AATCTAACAAAATCTCACCTG-3′) were used; these primers amplify a 181-bp fragment of the N-terminal region of the capsid gene (ORF 2) of NoV. RNA samples, along with 100 U of M-MLV reverse transcriptase (Invitrogen™ Life Technologies, Eugene, OR, USA) and the primer NoV SWNV₂, were used for cDNA synthesis, and RT-PCR was performed as described by Silva et al. [6]. Positive samples were purified using a GFX kit (GE Healthcare, Little Chalfont, UK) and subjected to DNA sequencing performed on an ABI 3500 sequencer with the corresponding primers, and the nucleotide sequence was genotyped using the Norovirus Genotyping Tool (RIVM) <http://www.rivm.nl/mpf/typintool/norovirus>) and CLC DNA Workbench 6.0.

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Results

NoV was detected in six samples (66.6 %) by RT-PCR, including 50% of the males ($n = 2$) and 80% of the females ($n = 4$), and all positive animals were asymptomatic at the time of collection. All NoV-positive samples were subjected to sequencing. Nevertheless, only three of the six samples were successfully sequenced: strain M1507/14 (KX356088), strain M1102/14 (KU587790) and strain M1510/14 (KU587791). Using the NoV Genotyping Tool, all three samples were characterized as genotype GII.11. The *Panthera onca* NoV GII.11 stool sequences were 100% identical to each other at the nucleotide level and 97.2% identical to representative swine GII.11 strains (AB126320 and EU193659) (Supplementary Material)

Discussion

The most common viruses that affect domestic cats have also been found in jaguars [2]. The sample data set presented here had already been tested for the presence of feline leukemia virus and feline immunodeficiency virus [7], but the occurrence of co-infections were not considered in the present study. NoVs have been reported to cause infection in carnivores such as captive lion cubs and has been associated with death caused by hemorrhagic enteritis [8].

Animals in the wild are important for the onset of outbreaks, often serving as reservoirs from which zoonotic pathogens can spread. The emergence of emerging infectious diseases in wildlife is mainly related to the destruction of their habitats [9] and human incursion into natural environments, exposing these animals to pathogens through contact with humans and domesticated animals [2].

The main mode of transmission of NoV is fecal-oral contact through contaminated food and water, but it can also occur through direct contact with contaminated environmental samples. Infected individuals can shed the virus for up to 3 weeks and remain asymptomatic, favoring the infection of other species [10, 11]. NoV is resistant to many environmental factors, and its minimum infectious dose is low, allowing the virus to spread through droplets, fomites, and environmental contamination [12].

Genogroup II.11 NoV is associated with humans and pigs and has also been detected as a groundwater contaminant in Korea [13] and shellfish in Japan [14]. Infection of jaguars could indicate environmental contamination due to the lack of basic sanitation in several municipalities in Brazil, and different genogroups of NoV have been detected in water samples from the surfaces of rivers as well as from treated and untreated sewage [11, 15].

Many pathogens causing intestinal infections can be excreted via the feces of infected individuals, and these include enteric viruses such as NoVs, which represent a threat to public health in water-associated environments [16]. Viruses are not inactivated through the treatment of domestic sewage, which is then dumped directly into rivers [13]. In the urban area of the municipality of Cáceres-MT, solid waste and sewage are not properly treated and are discharged into waterways and regional rivers [17].

The surveillance of NoVs is important for public health, since there is the possibility of zoonotic transmission and indirect infection via the food chain [18]. The feeding habits of jaguars include livestock predation, but 48% of their prey consists of wild species such as capybaras, peccaries, deer, and raccoons [19]. There have been no reports of human infections by animal NoV strains; however, animals infected with viral strains that are typically associated with a certain species might develop coinfections with other NoV strains, resulting in the emergence of recombinant agents with altered virulence [18], as has been observed with human and porcine rotaviruses [4].

Due to jaguar population decline, health and epidemiological studies have been conducted involving free-living animals in Pantanal; according to these data, contact with pathogens might exacerbate other threats faced by this species, affecting the health of these and other populations. This study demonstrates the occurrence of NoVs in wild jaguars, suggesting the presence of this virus in the environment. Further studies should be conducted to elucidate possible sources of contamination and the involvement of other species in the region.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interests exist.

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

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