Article

Group A rotavirus gastroenteritis in orphaned *Leopardus tigrinus* and *Leopardus pardalis* (CARNIVORA: FELIDAE) in the Eastern Amazon

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Abstract

Rotaviruses are highly infectious and typically transmitted by fecal-oral route via in the tropics and leading the cause of diarrheal deaths in children of developing countries, besides causing significant economic impacts like neonatal disease agents of domestic animals. This present report aims to present the clinical and diagnostic findings of two confirmed cases of rotavirus (RV) infection in orphaned *Leopardus tigrinus* (Schreber, 1775) and *Leopardus pardalis* (Linnaeus, 1758), the first register of the infection by group A rotavirus in these species. Both felids were rescued in the Pará State Amazon Brazil by the IBAMA (the Brazilian Institute of Environment and Renewable Natural Resources), and treated by veterinarians into intensive care ward in a public Environmental Park of Belém city. After the adaptation period to the quarantine, these animals showed non-specific symptoms of acute fulminant gastroenteritis. Rotavirus group A antigen was identified in blood and faecal samples of *L. tigrinus* analyzed by immunochromatography (ICG) and immunoassay methods (ELISA) at the Virology Laboratory of the Institute Evandro Chagas. The animals died within few days during the clinical exacerbation unresponsive to current treatment, its necropsies and histopathological analysis were performed in the Laboratory of Veterinary Pathology of the Federal Rural University of Amazonia (UFRA). Despite the compatible pathologic findings of rotavirus infection in both animals, the atypical hemorrhagic character was a curious finding, considering the presumed etiology.

Keywords: Group A rotavirus gastroenteritis; Emerging zoonotic viral diseases; *Leopardus tigrinus* and *Leopardus pardalis*; Endangered Neotropical Rain Forest Felids; Rehabilitation of injured or orphaned native wild cats.

Introduction

Rotaviruses are diseases caused by rotaviruses (RVs) that make up a large population genetically diverse of double-stranded RNA viruses into an

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individual genus of the Reoviridae Family, classified into groups, subgroups and serotypes [1, 2]. According the antigenic characteristics, rotavirus (RV) strains can be classified into groups of A-G and subgroups (SG) I, II, I + II, non-I e non-II [3]. SG II is prevalent among humans, while SG I is more frequently detected in animals [4, 5]. Groups like G3 are common found in humans and in different animals like cats, dogs, monkeys, pigs, rabbits and horses [6].

RVs are icosahedral and non-enveloped viruses protected by an inner and outer capsid layer, made up of 32 capsomers and 11 segments of ds RNA (16–21 kbp) [7, 8, 9]. Genetically, rotaviruses are diverse; its different gene segments are typically distributed across various animal species, suggesting the existence of host species barriers and host range restrictions according to the reservoir species, as well as the occurrence of homologous genes with epidemiological importance in zoonotic infections [1, 10]. Additionally, there are reports of rotavirus samples isolated from humans and animals, including birds and mammals, that share genetic and antigenic characteristics of virus samples from heterologous species, although combinations considered unusual are less frequent [11, 12, 13, 14].

Clinically, enteric viruses caused by group A RVs and other viruses such as coronavirus are reported in domestic cats and dogs, which promote gastroenteritis with unspecific symptoms, including vomiting and acute watery dehydrating diarrhea [11, 12, 15, 16].

RVs are important gastroenteritis agents in children under five years of age and neonatal diarrhea associated with mortality worldwide, with a limited number of deaths in developed countries and, in several wild and domestic animal species, etiologically causing delayed growth, increased susceptibility to other diseases, and increased mortality [2, 17, 18]. In addition, rotaviruses have been found as a cause of gastroenteritis in domestic cats in several countries, since the 1970s [19, 20, 21].

The case study presents the first confirmation of RV infection in wild cats of the species *L. tigrinus*, the smallest wild Brazilian felid and one of the least known among neotropical felids and, *L. pardalis* in the Amazon [22]. Aiming to discuss the evolution of the disease course in these carnivores in captivity and the aspects of transmission involving the case, postulating the origin of rotaviruses from domestic cats, also considering that the both orphaned felids

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presented symptoms of acute infection by enteric viruses in the rehabilitation quarantine. In addition, it intends to discuss this occurrence, thus contributing to the studies of the evolution and epidemiology of rotavirus infections, by recording two clinical cases of simultaneous occurrence and horizontal transmission.

RV infections in domestic and wild cats may be related to the occurrence of strains homologous to strain AU228, infective for humans [23]. The disease in humans can be considered emergent, resulting from the adaptation of feline RVs, involving the genetic and evolutionary interaction of strains that, currently, with the advent of new faster diagnostic tools, are classified based on the complete genome sequencing, enabling phylogenetic reconstruction studies of RV genotypes [24, 25, 26, 27].

The existence of enteric viruses in animals seems as a potential reservoir for infections in humans and, consequently, the study of these viruses in animals is considered the key to gaining a greater understanding of the evolution of these agents, as well as understanding the process of breaking down interspecies barrier [28, 29, 30, 31].

RVs infections can evolve asymptomatically, but hosts can eliminate the virus in their feces during a period of undetermined transmissibility, serving as a possible reservoir for viruses in their natural habitat [21, 32]. Although the natural transmission of RV between animal species is still the subject of studies and not sufficiently proven yet, the possibility should not be overlooked in the development of epidemiological studies [33].

Studies on the frequency of RVs and other enteric viruses in domestic and wild animals in the Amazon region are scarce when compared to the biodiversity of this region and in view of the present anthropic transformations of the Amazon biome [34, 35, 36]. This, among other records, is important in the evaluation of the epidemiological profile of enteric viruses associated with infections in animal populations *in situ* on preserved habitat and from areas degraded by anthropic action [35, 36, 37, 38].

Case report

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Use of the Federal Rural University of Amazônia (CEUA-UFRA, authorization No . 034/2014).

The IBAMA environmental agency agents rescued and sent to the Veterinary Sector of the Mangal das Garças Environmental Park (SV-PAMG), located in the Municipality of Belém (01° 27’ 21” S and 48° 30’ 16” W), State of Pará, Amazon, Brazil, two orphaned male kittens of species *L. tigrinus*, endangered species and *L. pardalis*, both species with wide geographic distribution in Central and South America [37].

The *L. pardalis*, three months old, was kept illegally in a residence in the Municipality of Altamira in the State of Pará (03° 12’ 12” S and 52° 12’ 23” W) [37]. The animal was kept in the SV-PAMG together with an orphaned *L. tigrinus* at the same age, which was received 2 months before, by IBAMA environmental agency agents from the city of Chaves, Marajó Island, Pará state, Brazil (00° 09' 36" S, 49° 59' 16" W).

At the SV-PAMG restrictive quarantine protocols for long-term care facilities were implemented considering the physical and biological needs of the two species. A nutritional and behavioral assessment form was prepared for the daily register during the quarantine and captivity of the cubs.

Both animals were clinically evaluated during all the period of quarentine, and after a period of two months of adaptation to quarantine and management in captivity, the *L. pardalis* began to present episodes of postprandial emesis and rapid progression of cachexia and dehydration. A week later, watery diarrhea appeared yellowish and had a strong odor. During this period, the specimen of *L. tigrinus* also started to show diarrhea.

During the symptomatic phase, the animals received daily support therapy with fluid therapy (electrolytes and vitamin complexes), symptomatic treatment with administration of bromopride and loperamide hydrochloride and chemotherapy treatment with enrofloxacin and metronidazole.

Twelve days after the onset of clinical signs, the *L. pardalis* died, but the necropsy findings were insufficient to determine the specific cause of the infection. Concomitantly, the *L. tigrinus*, still undergoing intensive clinical treatment, was subjected to blood testing procedures. The samples were collected in blood collection tubes (Vacutainer®) containing ethylenediaminetetraacetic acid (EDTA) for hemogram and feces (collection

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flask for clinical analysis) for enterovirus research at the Virology Laboratory of the Instituto Evandro Chagas, Secretaria de Vigilância e Saúde do Ministério da Saúde (IEC/SVS/MS), State of Pará.

The analytical procedures were carried out at the Institute Evandro Chagas (IEC / SVS / MS), and the stool analysis being specifically referred to the Virology Section of the IEC / SVS / MS. The feces samples were preserved at -20ºC until their processing by the immunochromatographic (ICG) and immunoenzymatic (ELISA) methods, starting from the preparation of suspensions (5% and 10%, mass *versus* volume) and execution strictly as prescribed by the manufacturers. Synthetically, the ICG involved the commercial kit Rota-Strip© (CORIS, BioConcept, Glemboux, Belgium), containing nitrocellulose strips sensitized by strip with polyclonal antibodies directed to group A RV proteins. The suspension under test and a complex involving antibodies monoclonal specifics for RVs coupled with particulate gold, migrate together via passive diffusion in the ribbon, resulting in a positive reaction expressed by the appearance of a line with a bluish-red color. As for the ELISA, was used the commercial kit RIDASCREEN© (R-Biopharm AG, Darmsdat, Germany), which consists of the use of microplates previously sensitized with specific monoclonal antibodies to the viral protein VP6. In a later stage, the same antibodies were used, although specific enzymatic conjugation. Group A RV antigen was identified in the fecal sample of *L. tigrinus*, which also died 13 days after the appearance of the first symptoms. The complete blood count of this animal showed normocytic and normochromic anemia (Ht 21.1%; Hm 3.970.000/mm3; Hb 7.8), with reactive leukocytosis (23.340/mm3).

Immunochromatography (ICG) and immunoassay (ELISA) of this animal's stool sample diagnosed the presence of the RV group A antigen. Based on these results, the animal was immediately subjected to targeted treatment with nitazoxanide and, after a thirteen-day period of convalescence of diarrhea recurred, the animal died.

Parasitological stool examinations for both were negative for the presence of intestinal endoparasites. In addition, feces samples were tested by different conventional molecular methodologies for RVs, however, due to their low viral load; positive samples could not be characterized.

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Both animals following death were necropsied in the Laboratório de Patologia Animal da Universidade Federal Rural da Amazônia (LABPAT-UFRA). In the ectoscopic examination, *L. pardalis* showed signs of emaciation, pale mucous membranes (Figure 1A) and mucous-shaped stools adhered to the perianal region (Figure 1B). In the small intestine, the mucosa was hyperemic and swollen, and there was an abundance of mucus (characteristic of catarrhal enteritis).

L. tigrinus also presented cachexia, conjunctival mucosa palor (Figure 2A), in addition to a citrus-yellow liquid in the abdominal cavity, slightly cloudy (Figure 2B), containing small fibrinous lumps (characteristic feature of serofibrinous peritonitis). The cytology of this liquid confirmed peritonitis due to the prominent presence of neutrophils and an increase in macrophages (Figure 2C). In the small intestine, changes predominated in the jejunum, with a notable presence of mucus mixed with hemolysed blood (characteristic of hemorrhagic catarrhal enteritis) (Figure 2D).

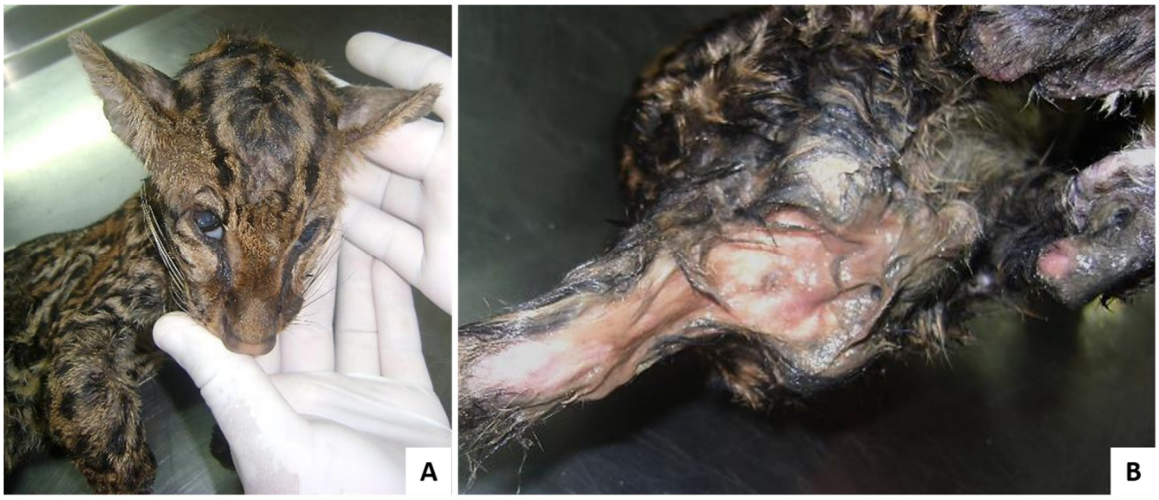

Figure 1. A. *L. pardalis* depleted state, dehydrated and pale conjunctiva **B.** Very humid perianal region due to mucoid diarrhea.

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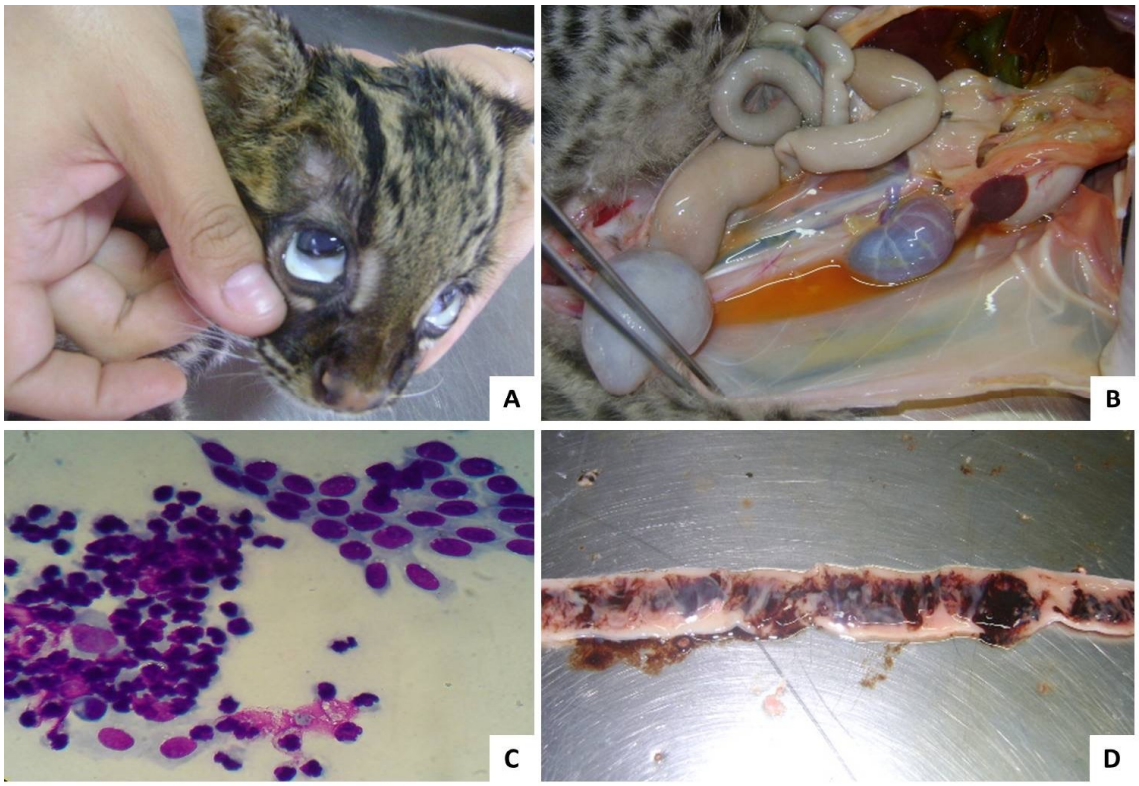

Figure 2. A. *L. tigrinus* presenting conjunctival mucosa palor. **B.** Increased citrus yellow fluid into the abdominal cavity. **C.** Peritoneal cytology, showing mesothelial cells and increased neutrophils. **D.** Duodenum with mucus-hemorrhagic exudate.

Fragments of the intestines of both animals and other organs were collected for histopathological analysis. The duodenal villi of both animals showed an increase in desquamation and more dilated intestinal crypts. In the ileum of *L. tigrinus* the villi were shorter with noticeable desquamation and the dilated crypts showed necrotic remains, together with erythrocytes, in addition to the regeneration stage of villus epithelial cells. Histologically, no major changes were observed in the other organs.

In view of the suspicion of an outbreak of human RVs in several states in Brazil and, despite the confirmation of the diagnosis of human RVs gastroenteritis in the samples of *L. tigrinus*, it was not possible to start an epidemiological investigation of the cases. However, exist the register that the quarantine animal care keeper of SV-PAMG was the owner of a domestic cat that had been hospitalized a month before the onset of the symptoms of wild cats in a veterinary clinic in the city of Belém. According to veterinarians of that

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clinic others similar cases of domestic cats with diarrhea, vomiting and dehydration have also been reported, and these animals recovered after treatment of non-specific gastroenteritis based on antibiotic therapy (enrofloxacin®) fluid therapy (electrolytes and vitamin complexes). Also in the same period that wild cats had gastroenteritis symptoms, positive cases of diarrhea with clinical suspicion of human RVs disease were reported by workers of SV-PAMG.

Discussion

L. tigrinus, a species considered at risk of extinction by the International Union for Conservation of Nature (IUCN), and *L. pardalis* are neotropical felids that suffer constant anthropic pressure mainly due to habitat loss and fragmentation caused by agricultural expansion [39]. Cubs of these and other wild mammals are frequently rescued by the environmental inspection bodies in the Amazon and, destined for care in Brazilian fauna conservation institutions [37, 38]

In this context, it has been reported that the direct contact of these animals with humans and other species, including domestic animals, can facilitate the spread of emerging diseases, affecting new hosts and, notifications for new geographic occurrences, as reported by infections by parasites, coronavirus, retrovirus, feline leukemia virus and feline immunodeficiency virus in wild cats, among other pathogens [40, 41].

Although the reports still controversial in terms of admitting the transmission between RV species under natural conditions [1, 17]. reinforcing the need for more studies have been developed in this area, based mainly on the complete genetic sequencing of the samples, what would can range the discussions about the occurrence or not of heterologous infection [42, 43, 44].

Evidence of interspecies transmission and genetic rearrangements between human and animal RVs has been reported [36]. Some animal species seem to contribute frequently to this antigenic/ genetic diversity found in human rotavirus infections, presumably because of close interactions with other animal species, including domestic ones [1, 36].

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Group A RVs are the most important epidemiologically because they are associated with the higher number of infections [45]. However, groups A, B and C RVs have infected humans and other mammals [35, 45] Groups D, F and G RVs were found only in birds [46, 47]. However, there are reports of avian group A RVs, which generally fall into a separate genotype and show different electrophoretic patterns from viruses that infect mammals, suggesting that transmission of these avian viruses to mammals would be unlikely [47, 48]. This conclusion could confirm that there is no obvious relationship of risk of transmission from birds of the PAMG to the two quarantined wild cats.

However, it is worth mentioning that group A RVs of avian origin have already been found in other bird species such as: chickens, ducks, pigeons, ostriches, pheasants and other wild birds [47, 48]. In Brazil, these RVs were first identified in broiler chicken feces samples and, since then, few studies have been developed in the national territory aiming at the detection of these agents in birds [49].

In mammals, there are records of detection around 10% of pigs infections caused by group A RVs in the Metropolitan Region of Belém, with predominance of G3 and G5 genotypes, P genotypes following: P[13]/[22] and P[23] and its most common combinations following: G3P[13]/[22];G3P[23] and G5P[13]/[22] [50]. In developing countries, including Asian and sub-Saharan African countries, strains of RVs of human origin with unusual combinations of the G and P types by recombination with RVs strains of animals [36, 51].

Although pigs and cattle carry group A RV infection sub-clinically at low frequency, strategies are urgently needed to better understand the dynamic of zoonotic risk of transmission to humans [52, 53]. In addition, the porcine genogroup E RVs are considered uncommon and their pathogenesis is not studied well and`the newly distinct porcine genogroup H RVs, recently, in Japan, was confirmed the infection by H RVs causing diarrheia in pigs and infecting humans in Japan, China, and Bangladesh [52, 53].

Samples of neonates and children with acute diarrhea in the metropolitan region of Belém, where the circulation of group A RVs is recorded, had present in the results of analysis the NSP4 and VP4 genes similar to those of swine origin, suggesting a possible transmission between species showing the close relationship between human and animal origin [35, 48, 54, 55]

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These viruses are often isolated in pediatric infections and, although the transmission chain is still not well understood, some studies show that these viruses are present in wastewater, causing recurrent infections, especially in emerging countries [21, 56, 57, 58]. Admittedly, rotavirosis has a higher occurrence in young animals and many species of mammals and birds and can cause symptomatic and asymptomatic infections in cats [59, 60]. Most infections are subclinical, which suggests that the immune system of the infected animal is able to stop the evolution of the disease before the symptoms manifest [61]. However, orphaned animals under varying stress conditions have a greater immunological susceptibility, which favors the development of infections and their complications in co-infections [62]. Nevertheless, the expanding landscape of RV genotypes of has changed substantially in the last decades, including the study of autoimmune triggering to clinical spectrum of this infection, which is known to be much broader than acute diarrhea [63].

RVs may be the primary cause or be associated with other enteropathogens, showing that the genetic diversity of such viruses seems to be more frequent in developing countries, probably due to low levels of hygiene, depressed immune defenses, concomitant parasitic infections, malnutrition, in addition to the close relationship between man, domestic animals (canines and felines) and domesticated animals (pigs and birds) [21, 34, 64, 65].

The pathophysiological mechanism of gastroenteritis associated with RV is clearly multifactorial, involving a role of several mechanisms and a short incubation period like malabsorption secondary to enterocyte destruction, stimulation of the enteric nervous system (ENS) and villus ischemia [66]. All these factors contribute to the pathogenesis of diarrhea [66, 67]. In addition, to an extremely short incubation period, the enterotoxigenic potential of NSP4 of Rotavirus, designated as the first viral enterotoxin, has demonstrated the role of NSP4 association in various mechanisms investigated up till now [21, 67, 68].

The targeted treatment with nitazoxanide, indicated for the treatment of viral gastroenteritis caused by Rotavirus and Norovirus in humans, resulted in partial recovery of *L. tigrinus* with the production of more pasty stools during the thirteen days of treatment [69, 70, 71]. However the disease promoted a severe condition of physical depletion in the animal in question and evolution to death

that may be related to the serum levels of ammonia and other toxins associated with the recurrence of viral infections [66, 67, 68].

The epidemiological investigation of the present case is limited, because in parallel with the diagnosis of rotavirus infection in quarantined orphaned cats, it was not possible to perform specific tests that could assist in determining which pathway was responsible for contagion with RV serotype A, if other local animal species they would be infected and could act as asymptomatic carriers and/or if the transmission would have occurred through contact with contaminated water or fomites [59, 72].

According to reports by some veterinarians from veterinary clinics in Belém, PA, clinical cases of domestic cats that showed clinical symptoms compatible with rotavirus disease were recorded in the period when the two wild cats developed the symptoms, as well as symptomatic human cases reported by the local health units [73].

However, this is the first confirmed case of group A RV in felines of the species *L. tigrinus* and *L. pardalis*, which requires complementary studies, particularly on the genetics of the agent, aiming to identify the subgroups involved in the transmission of the virus to wild cats that can infect domestic and human felines in the Amazon region, mainly due to the high potential for dissemination and the wide variety of strains of the circulating virus, favoring seasonality due to climatic, environmental and basic sanitation factors in an indeventible area of the Amazon.

Conclusion

The results obtained allow us to conclude that the RVs can infect wild cats, promoting a rapid course with non-specific symptoms and evolving to death. This work complements other reports of RV in animals and suggests the need to conduct an epidemiological survey to understand the chain of transmission of rotavirus infections among susceptible hosts and to better determine the origin and evolution of these agents. Showing that additional research should be planned for the continuous monitoring of animal species for the detection of new complete genomes of RVs.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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