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Short Communication

RVA in Pet, Sheltered, and Stray Dogs and Cats in Brazil

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A B S T R A C T

Rotaviruses species A (RVA) are etiological agents of diarrhoea and are considered zoonotic viruses; yet the epidemiology of RVA among pet animals is largely unknown. RVA was detected in 38 of 308 faecal samples (12.3%) from pet, sheltered, or stray dogs and cats in 2 municipalities of Rio de Janeiro state, Brazil. The results indicated that these viruses are common in canine and feline populations and underscore the importance of improved monitoring of common pathogens in companion animals, with increased awareness of the potential for interspecies transmission events.

As companion animals, dogs (*Canis lupus familiaris*) and cats (*Felis catus*) have intense contact with humans sharing the same environment. However, animals can be a source of pathogens that are capable of infect humans, including rotavirus.[1] Rotaviruses (RVs) are classified into the *Rotavirus* genus of the *Reoviridae* family and comprise 12 species (*A-L*).[2] To date, species A (RVA) has been the most common RV detected among canines and felines.[3] Nevertheless, species C (RVC) and I (RVI) have also been identified in dogs and cats.[4-7]

Although there are strong indications of the zoonotic potential of canine and feline RVA strains,[8] the epidemiological behaviour of these viruses remains unknown. Considering the extensive contact between humans, dogs and cats, it is important that this knowledge be expanded. In this study we aimed to detect RVA infections in faecal samples from domestic canines and felines, with or without diarrhoea, pet, sheltered or stray, to assess the prevalence of these infections.

Three hundred eight faecal specimens from 205 dogs and 103 cats were collected between May 2018 and June 2019. Samples were obtained from animals with or without diarrhoea in the cities of Rio de Janeiro and Campos dos Goytacazes, in the state of Rio de Janeiro, Brazil. Sample collection was performed, from each animal individually, by trained veterinarians, taking the necessary precautions to avoid cross-contamination. The stool from pet animals were obtained from animals that were being treated in veterinary clinics and, the sample collection was performed by attending veterinarians; samples of sheltered animals came from different shelters and sample collection was performed by one of the veterinarians in our group. Samples of stray animals were obtained from the Center for Zoonosis Control, and the collection was carried out by our collaborators, veterinarians from the center.

This study was approved by the Animal Research Ethics Committee of the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (Approval number 130/18; Approval date 20 September 2018).

Stool suspensions were prepared in 10% (w/v) phosphate-buffered saline (PBS; pH 7.2) and then centrifuged at 2,500 $\times g$ for 5 minutes. Nucleic acid was extracted from 300 μL of the supernatant using the guanidine isothiocyanate-phenol–chloroform method. RVA RNA was detected by reverse-transcription PCR (RT-PCR) and semi-nested PCR amplification of a target fragment of the VP6-encoding gene, using the protocol described previously[9,10] that generate products of 370 and 220 bp, respectively. For positive and negative PCR controls were used, the cell cultured simian rotavirus strains SA11 and PBS, respectively. PCR products were separated by 1.2% (w/v) agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light. A 100-bp DNA ladder (Ludwig Biotec) was used to determine molecular size.

The semi-nested amplified products (220 pb) from 22 RVA-positive samples were sent to Macrogen (Seoul, Korea) for sequencing. Overlapping sequences were assembled and edited using the Lasergene software package (DNASTAR). Phylogenetic analysis was performed with MEGA software (v.7.0.14; https://www.megasoftware.net/).[11] A dendrogram was constructed using the maximum likelihood method based on the Kimura 2-parameter model. Statistical significance was estimated by bootstrap analysis with 1000 pseudoreplicates. Sequences were compared to reference RVA strains from GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/).Generated sequences were deposited into GenBank under accessions numbers OL870493-OL870514 (Fig 1).

Overall, 38 (12.3%) samples were positive for RVA. Positive samples were detected at both sites, among animals of all ages, pet, sheltered and stray (Table 1). Of the 205 canine faecal samples, 25 (12.2%) were positive for RVA. Prevalence was 11.7% (11/94) and 12.6% (14/111) among dogs from Rio de Janeiro and Campos de Goytacazes, respectively, and was significantly elevated among symptomatic

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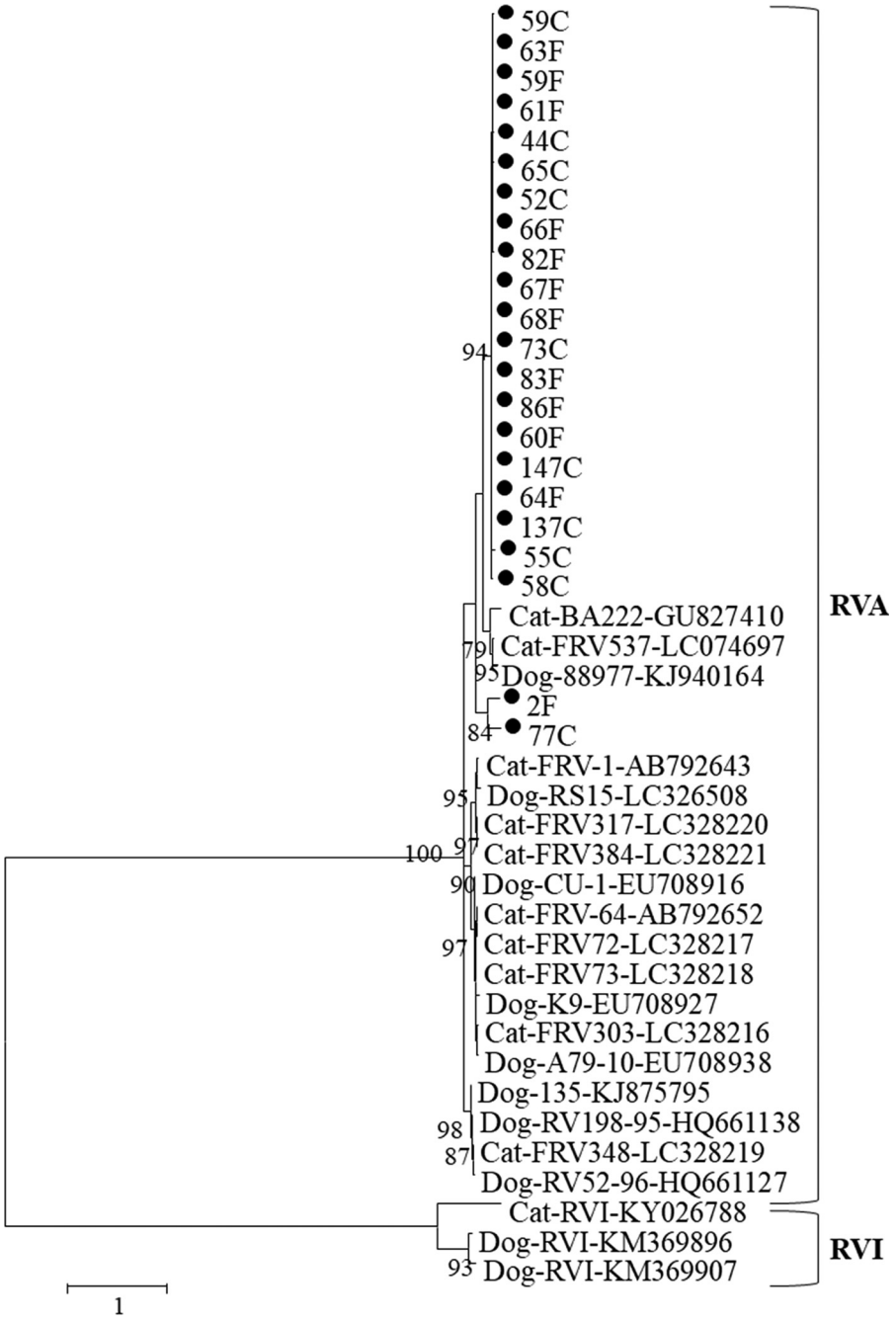

Fig 1. Dendrogram constructed from partial sequences of the VP6 of canine and feline RVA strains. Distances were corrected using the Kimura 2-parameter model and the dendrogram was constructed using the maximum likelihood method. Statistical support was provided by bootstrapping 1000 pseudoreplicates. Bootstrap values above 75% are given as branch nodes. Genbank reference strain accession numbers are shown next to the strain identification. Strains detected in this study are indicated by black circles. RVI strains were used as outgroup.

(n = 23) than asymptomatic (n = 2) animals ($P = .006$). Prevalence was similar between pet and stray dogs ($P = .098$). Age distribution of dogs disclosed 76 puppies (≤1 year old), 79 adults (>1-7 years old), and 20 elderly (>7 years old) animals. The ages of 30 dogs were not reported. Among the 25 RVA-positive dogs, 8 (32%) were puppies, 10 (40%) were adults, and 2 (8%) elderly; the ages of 5 animals could not be determined.

Thirteen (12.6%) feline samples were positive for RVA: 12 (92.3%) from Rio de Janeiro and 1 (7.7%) from Campos de Goytacazes. Rotavirus was detected among cats with and without diarrhoea, pet, sheltered and stray, with no statistically significant differences. Overall, 29 cats were kittens (≤1 year old), 57 were adults (>1-7 years old), and 6 were elderly (>7 years old). The age of 11 cats was not reported. Among the 13 RVA-positive cats, 3 (23.1%) were kittens, 7 (53.8%) were adults, and 1 (7.7%) was elderly; the age of 2 animals could not be determined.

The prevalence and clinical significance of canine and feline RV infections are incompletely understood, as only a limited number of epidemiological studies have been published. Previous studies have reported 0.7%-43% RVA prevalence among dogs[16,15,12,13,17], and 3%-50% among cats.[16,14,13] In general, these studies were conducted among pet or sheltered animals, so RVA prevalence among stray animals is unknown. Our results revealed a high prevalence of RVA in canines and felines, and in pet, sheltered and stray animals. In the case of stray animals, viral shedding was more common among canines. Although viral detection was more frequent among diarrheal

Table 1
Characteristics of RVA-positive dogs and cats

Host	Collection site	N° tested samples	N° of positive samples/Total tested								
			Clinical status		Household situation		Age				
			Diarrheal	Asymptomatic	Pet	Sheltered	Stray	Juvenile	Adult	Elderly	Unknown
Dogs	Rio de Janeiro	94	10/70	1/24	11/90	0/1	0/3	5/29	2/27	2/20	2/18
	Campos de Goytacazes	111	13/70	1/41	0/0	0/0	14/111	3/47	8/52	0/0	3/12
Total		**205**	**23/140**	**2/65**	**11/90**	**0/1**	**14/114**	**8/76**	**10/79**	**2/20**	**5/30**
Cats	Rio de Janeiro	62	7/47	5/15	7/37	5/23	0/2	3/22	6/23	1/6	2/11
	Campos de Goytacazes	41	0/30	1/11	0/0	0/0	1/41	0/7	1/34	0/0	0/0
Total		**103**	**7/77**	**6/26**	**7/37**	**5/23**	**1/43**	**3/29**	**7/57**	**1/6**	**2/11**
Total		**308**	**30/217**	**8/91**	**18/127**	**5/24**	**15/157**	**11/105**	**17/136**	**3/26**	**7/41**

The values in bold represent the total for each category.

animals, a direct association between RVA excretion and diarrhoea was not possible because other enteropathogens were not investigated.

Although canine and feline RVA (CRV and FRV, respectively) infections are usually subclinical or mild[18,3], they deserve attention as potential sources of human disease.[8] Human RVA strains with genetic homology with CRV and FRV have already been detected;[19-21] RVA with characteristics of human strains were also identified in canines and felines,[23,22] suggesting direct interspecies transmission. In addition, supposedly chimeric human-canine and human-feline strains have been identified in humans, suggesting gene exchange between such strains that may potentiate interspecies infections.[24-28] Some of these strains could eventually become epidemiologically relevant. Consequently, improved monitoring of common pathogens in companion animals, with increased awareness of the potential for interspecies transmission events is needed.

Author Contributions

Material preparation, and analysis were performed by PSF and CAMS. The first draft of the manuscript was written by PSF. Data collection was performed by FAM, MFNR and CEPFT. GSM and CEPFT contributed to the study conception and design, and to the reviewing and editing of the manuscript. NS was responsible for the conception and design of the study, contributed substantially to data analysis and interpretation, and critically revised the manuscript for important intellectual content. The final manuscript has been reviewed and approved by all the authors.

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