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Identification of enteric viruses circulating in a dog population with low vaccine coverage

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ABSTRACT

Although the use of vaccines has controlled enteric diseases in dogs in many developed countries, vaccine coverage is still under optimal situation in Brazil. There is a large population of nonimmunized dogs and few studies about the identification of the viruses associated with diarrhea. To address this situation, stool samples from 325 dogs were analyzed by polymerase chain reaction for the detection of common enteric viruses such as *Canine adenovirus* (CAdV), *Canine coronavirus* (CCoV), *Canine distemper virus* (CDV), *Canine rotavirus* (CRV) and *Carnivorous protoparvovirus 1* (canine parvovirus 2; CPV-2). At least one of these species was detected in 56.6% (184/325) of the samples. The viruses detected most frequently in either diarrheic or nondiarrheic dog feces were CPV-2 (54.3% of the positive samples), CDV (45.1%) and CCoV (30.4%), followed by CRV (8.2%) and CAdV (4.9%). Only one agent was detected in the majority of the positive samples (63%), but co-infections were present in 37% of the positive samples and mainly included CDV and CPV-2. The data presented herein can improve the clinical knowledge in regions with low vaccine coverage and highlight the need to improve the methods used to control these infectious diseases in domestic dogs.

Introduction

Gastrointestinal disorders are frequently reported in companion animal clinics as leading to severe dehydration and death in South America.[1,2] They can have bacterial, parasitic or viral etiologies.[3] Viruses associated with enteric illnesses in dogs are an important cause of mortality in nonprotected populations.[2] Among these, canine parvovirus (CPV-2) and canine coronavirus (CCoV) are considered the most common viral enteric pathogens in dogs worldwide.[4–8] Canine distemper virus (CDV) is endemic to South America and is frequently associated with enteric disorders.[8–11] Canine adenovirus type 1 (CAdV-1) is commonly linked to hepatitis but was also associated to severe gastroenteritis including vomiting and diarrhea.[12] The canine rotavirus (CRV) is an unusual

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enteric pathogen in dogs but is important due to its zoonotic potential.[13,14]

The search for multiple pathogens in fecal samples from dogs can mirror common exposure but can also show interactions between pathogens determining or aggravating disease.[15,16] Moreover, there is a lack of research searching for multiple viral pathogens in dogs, which could uncover the real etiology of canine gastroenteritis. Therefore, the present study aimed to verify the frequency of canine enteric viruses (CPV-2, CCoV, CDV, CAdV1 and CRV) in stool samples from dogs.

Materials and methods

Ethics and sample collection

Fecal samples were collected from the rectal ampullae of 325 dogs at veterinary clinics during 2008 and 2014 and stored at −20 °C. The animals sampled were from eight of the federal States of Brazil (Rio Grande do Sul, Santa Catarina, Paraná, Rio de Janeiro, São Paulo, Mato Grosso do Sul, Rondônia and Acre) (Fig. 1). The majority of the analyzed dog population was unvaccinated (282/325), 24 animals were vaccinated but with incomplete vaccine protocols and 19 received complete protocols (Table 1). Dogs defined as vaccinated received the complete vaccination protocol using three doses of polyvalent vaccine, while the ones defined as vaccinated with incomplete protocols received one or two doses. Polyvalent vaccines used in dogs in Brazil are constituted by CPV-2, CDV, CCoV, CAdV2, canine parainfluenza virus and *Leptospira* spp. with minor differences according the different manufacturers. The sanitary status was defined by the clinic during sample collection where 82/325 were apparently healthy, 77/325 presented enteric disease-associated signs and 166/325 had this information not determined. Table S1 describes associated-information about all dog samples analyzed in the present study.

The project was registered with the Ethics Committee on the Use of Animals (CEUA) of Universidade Federal do Rio Grande do Sul under protocol number #24984.

DNA/RNA isolation and reverse transcription (RT)

Fecal samples were diluted to 20% (w/v) in phosphate-buffered saline (PBS, pH 7.4) and stored at −80 °C for further analysis.

DNA was isolated from the supernatant using guanidine-isothiocyanate and a silica-based[17] commercial kit (NewGene Preamp, Simbios Biotecnologia, Cachoeirinha, RS, Brazil).

Total RNA was extracted using TRIzol® LS Reagent (Life Technologies®, Carlsbad, CA, USA). The cDNA was synthesized with SuperScript® III Reverse Transcriptase Kit (Life Technologies®, Carlsbad, CA, USA) using reverse primers for each target[9,18–21] in a total volume of 20 μL.

PCR

PCR and RT-PCR were performed using previously published protocols. The CPV-2 PCR protocol aimed to amplify 583 bp of the VP2 gene,[18] while the CAdV assay targeted 509 bp of the E3 coding region.[19] For CDV detection, RT-PCR was performed to amplify a fragment of 479 bp of the N gene, followed by nested PCR that aims to amplify a 286 bp fragment.[9] The CCoV protocol amplified 410 bp of the M gene,[20] and the CRV protocol targeted 1062 bp of the VP7 gene.[21] The PCR products were electrophoresed in 2% agarose gels, visualized under UV light and compared with a 100 bp molecular weight ladder (Ludwig Biotecnologia, Cotia, SP, Brazil).

PCR and RT-PCR positive samples were submitted for DNA sequencing (ACTGene Análises Moleculares Ltda., Alvorada, RS, Brazil) in order to confirm the identities of amplified viruses. PCR amplification products were purified using the NucleoSpin Extract II Kit (Macherey-Nagel, Düren, Germany). After, both strands were sequenced with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)

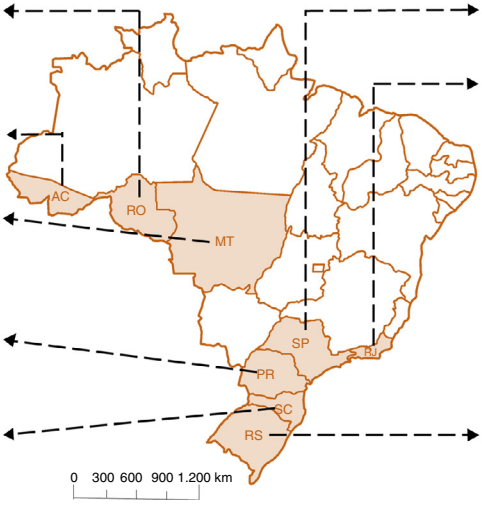
City	CPV-2	CDV	CCoV	CAdV	CRV	Total tested
Ji-paraná	1	0	2	0	0	3
TOTAL	1	0	2	0	0	3

City	CPV-2	CDV	CCoV	CAdV	CRV	Total tested
Rio branco	3	0	0	0	0	4
TOTAL	3	0	0	0	0	4

City	CPV-2	CDV	CCoV	CAdV	CRV	Total tested
Cuiabá	21	7	0	0	0	22
TOTAL	21	7	0	0	0	22

City	CPV-2	CDV	CCoV	CAdV	CRV	Total tested
Cambé	1	3	1	0	0	3
Londrina	17	10	3	0	2	39
Maringá	3	8	8	2	4	46
Tamarana	0	1	0	0	0	1
TOTAL	21	22	12	2	6	89

City	CPV-2	CDV	CCoV	CAdV	CRV	Total tested
Concórdia	0	0	0	0	0	5
Florianópolis	3	1	0	1	0	7
TOTAL	3	1	0	1	0	12



City	CPV-2	CDV	CCoV	CAdV	CRV	Total tested
São paulo	0	4	1	4	0	29
TOTAL	0	4	1	4	0	29

City	CPV-2	CDV	CCoV	CAdV	CRV	Total tested
Niterói	0	1	0	0	0	4
TOTAL	0	1	0	0	0	4

City	CPV-2	CDV	CCoV	CAdV	CRV	Total tested
Bagé	2	5	6	0	0	12
Cachoeirinha	0	1	0	0	0	10
Canoas	1	0	1	0	0	4
Caxias do sul	0	3	3	0	0	11
Glorinha	0	1	0	0	0	1
Gravataí	14	11	14	2	7	48
Lavras do sul	0	0	0	0	1	3
Novo hamburgo	0	0	0	0	0	1
Passo fundo	9	8	3	0	0	17
Pelotas	2	1	0	0	0	2
Porto alegre	18	15	7	0	0	34
Rio pardo	0	0	3	0	0	4
Santana do livramento	2	1	4	0	0	4
São francisco de paula	0	0	0	0	0	3
Santa maria	0	0	0	0	0	1
Taquara	1	0	0	0	0	1
Viamão	2	1	0	0	1	6
TOTAL	51	47	41	2	9	162

Fig. 1 – Map of Brazil. The map indicates the Brazilian federative state of origin of each sample describing the cities collected and number of positive samples for each evaluated virus. RS: Rio Grande do Sul; SC: Santa Catarina; PR: Paraná; SP: São Paulo; RJ: Rio de Janeiro; MT: Mato Grosso; RO: Rondônia; AC: Acre.

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