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Molecular detection of canine distemper virus (CDV), canine adenovirus A types 1 and 2 (CAdV-1 and CAdV-2), and canine parvovirus type 2 (CPV-2) in the urine of naturally infected dogs

Detecção molecular do vírus da cinomose canina (CDV), adenovírus canino A tipos 1 e 2 (CAdV-1 e CAdV-2) e parvovírus canino tipo 2 (CPV-2) em urina de cães naturalmente infectados

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Abstract

Canine distemper virus (CDV) causes systemic, respiratory, cutaneous, and neurological manifestations in dogs and other mammalians. Canine adenovirus A (CAdV) is divided in two types, CAdV-1 and CAdV-2, and cause infectious canine hepatitis and infectious tracheobronchitis, respectively. Canine parvovirus type 2 (CPV-2)-induced enteritis is one of the most common infectious cause of diarrhea in puppies. The aim of this study was to detect the urinary excretion of major viral pathogens that cause systemic infections in dogs. From December 2011 to December 2012, 41 urine samples were collected from dogs that presented systemic clinical signs of infectious diseases. The samples were evaluated by reverse transcription-polymerase chain reaction (RT-PCR) for CDV and by PCR for CAdV-1, CAdV-2, and CPV-2. RT-PCR amplified a partial fragment of the CDV N gene (287 bp) in 15 (36.6%) urine samples. PCR for E gene of CAdV-1 (508 bp) and CAdV-2 (1,030 bp) was positive in 4 (9.8%) and 1 (2.4%) sample, respectively; CPV-2 partial VP2 capsid protein gene (583 bp) was amplified in 6 (14.6%) samples. These results suggest that urine can be used as a clinical sample for the *ante mortem* diagnosis by molecular tools such as RT-PCR and PCR for CDV, CAdV-1 and 2, and CPV-2 systemic infections in dogs. Urinary excretion might be an important route for maintaining these viruses within the environment, and should be considered as a source of infection for healthy dogs.

Key words: Canine viral diseases, viruria, RT-PCR, PCR, coinfections

Resumo

O vírus da cinomose canina (CDV) é um *Morbilivirus* que causa manifestações clínicas sistêmicas, respiratórias, cutâneas e neurológicas em cães e outros mamíferos. O adenovírus canino A (CAdV) pode ser diferenciado em dois tipos, CAdV-1 e CAdV-2, e causam hepatite infecciosa canina e traqueobronquite infecciosa, respectivamente. Enterite pelo parvovírus canino tipo 2 (CPV-2) é uma das diarreias infecciosas mais comuns em cães, especialmente em filhotes. O objetivo deste estudo foi detectar a excreção urinária de alguns dos principais vírus que ocasionam infecções sistêmicas em cães. No período de dezembro de 2011 a dezembro de 2012 foram colhidas 41 amostras de urina de cães com sinais clínicos sistêmicos. As amostras foram avaliadas por reação em cadeia da polimerase precedida por transcrição reversa (RT-PCR) para o CDV e por PCR para CAdV-1, CAdV-2 e CPV-2. A RT-PCR

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amplificou um fragmento do gene N do CDV (287 pb) em 15 (36,6%) amostras. A PCR para o gene E do CAdV-1 (508 pb) e do CAdV-2 (1030 pb) foi positiva em 4 (9,8%) e 1 (2,4%) amostra, respectivamente; o gene VP2 da proteína do capsídeo do CPV-2 (583 pb) foi amplificado em 6 (14,6%) amostras. Estes resultados sugerem que a urina pode ser utilizada como amostra clínica para o diagnóstico *ante mortem* de infecções sistêmicas por CDV, CAdV-1 e 2 e CPV-2 por técnicas moleculares como RT-PCR e PCR. Adicionalmente, a excreção viral pela urina parece ser uma importante rota para a manutenção destes vírus no ambiente e deve ser considerada como fonte de infecção para cães saudáveis.

Palavras-chave: Doenças virais caninas, virúria, RT-PCR, PCR, coinfecções

Canine distemper virus (CDV) is a *Morbilivirus* that causes systemic, respiratory, cutaneous, and neurological manifestations in dogs and other mammalians (GREENE; VANDEVELDE, 2012). CDV is considered as one of the major causes of mortality of young and adult dogs in Brazil, impacting the local economy due to the therapy associated with the systemic effects of CDV (HEADLEY et al., 2012). Canine adenovirus A (CAdV) is differentiated in two types, CAdV-1 and CAdV-2, which are closely related genetically and antigenically. CAdV-1 and CAdV-2 are responsible for infectious canine hepatitis, characterized by corneal edema, and infectious tracheobronchitis, respectively (DECARO; MARTELLA; BUONAVOGLIA, 2008). Canine parvovirus enteritis is one of the most common infectious diarrheic disease of dogs, and is caused by distinct strains of canine parvovirus 2 (CPV-2, 2a, 2b, and 2c). The clinical illness of CPV-2 is more common in young pups that are not vaccinated, especially between 6 weeks and 6 months of age (GREENE; DECARO, 2012).

The clinical diagnosis of canine distemper, infectious canine hepatitis, infectious tracheobronchitis and canine parvovirus enteritis are often difficult due to the variability of clinical manifestations. The use of RT-PCR and PCR asseys to detect CDV RNA, and CAdV-1 and CAdV-2 DNA in the urine of dogs with clinical signs of these diseases has been reported (NEGRÃO; ALFIERI; ALFIERI, 2007; HEADLEY et al., 2013). Urine is easier to collect than other body fluids and is useful in the *ante mortem* diagnosis of distemper and other viral diseases (SAITO et al., 2006; HEADLEY et al., 2013). The current study evaluated the use of urine in RT-PCR and PCR assays for the detection of CDV, CAdV-1, CAdV-2, and CPV-2.

From December 2011 to December 2012, 41 urine samples from dogs were received at the Laboratory of Animal Virology, Universidade Estadual de Londrina, southern Brazil, to be evaluated for the presence of CDV. However, there is known associated description of clinical manifestations, immunological status, vaccination schedule, age, gender, or history of the animals. All samples were collected via cystocentesis or urethral catheterization. In addition to distemper, the samples were also evaluated by PCR assays for the detection of CAdV-1, CAdV-2, and CPV-2.

Nucleic acid were extracted from a volume of 300 µL of the urine sample by using the silica/guanidine isothiocyanate method (BOOM et al., 1990). Positive controls consisted of aliquots from commercial vaccines (CAdV-2 and CPV-2), and from previously described cases (CAdV-1 and CDV) (HEADLEY et al., 2013); nuclease-free water (Invitrogen™ Life Technology) was used as negative control.

The RT-PCR assay targeted a 287 base pair (bp) fragment of the N gene of CDV (FRISK et al., 1999), with modifications (SAITO et al., 2006). The amplification of CAdV-1 and CAdV-2 viral DNA was performed by using a PCR assay designed to detect the 508 bp and 1,030 bp fragments of the E gene of CAdV-1 and CAdV-2, respectively (HU et al., 2001). PCR assay was also performed to amplify the 583 bp fragment of the VP2 gene of CPV-2 (HONG et al., 2007). The PCR products were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide, and visualized under UV light. The primers used in the RT-PCR and PCR assays are given in Table 1. The partial nucleotide sequences of the CDV N gene, CAdV-1 E gene, and CPV-2 VP2 gene obtained were initially compared with those deposited in GenBank by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

Table 1. Primers used for the detection of viruria in dogs with systemic infections.

Virus	Primer	Sequence (5'-3')	Target gene	Amplicon size (bp)	References
CDV	p1 (F) p2 (R)	ACAGGATTGCTGAGGACCTAT CAAGATAACCATGTACGGTGC	Nucleoprotein	287	Frisk et al., 1999
CAdV-1 and CAdV-2	HA1 (F) HA2 (R)	CGCGCTGAACATTACTACCTTGTC CCTAGAGCACTTCGTGTCCGCTT	E3	508 and 1,030	Hu et al., 2001
CPV-2	Forward (F) Reverse (R)	CAGGAAGATATCCAGAAGGA GGTGCTAGTTGATATGTAATAAACA	VP2	583	Hong et al., 2007

F (forward); R (reverse).
Source: Elaborated by the authors.

The results of the demonstration of viruria are shown in Table 2. Of the 41 urine samples analyzed, 21 (51.2%) contained at least one of the viruses evaluated in this study. The RT-PCR assay for CDV N gene amplified the expected 287 bp fragment in 15 (36.6%) samples. CAdV-1 and CAdV-2 DNA were identified in 4 (9.8%) and 1 (2.4%) of the evaluated urine samples, respectively. CPV-2 was detected by PCR assay in 6 (14.6%) urine samples. Twenty (48.8%) samples were negative for all viruses evaluated. Of the 21 positive samples, viruria due to a single viral pathogen was observed in 18 (85.7%) of these. Concomitant detection of viral pathogens occurred in 3 (14.3%) urine samples. One of these demonstrated viruria for all four agents evaluated (CDV, CAdV-1, CAdV-2, and CPV-2); one sample was positive for CDV and CAdV-1, while concomitant CDV and CPV-2 viruria was observed in another sample.

Table 2. Molecular (RT-PCR or PCR) detection of viruria from 41 urine samples of dogs.

Virus	Infections		TOTAL
	Single	Mixed	
CDV	12	3	15
CAdV-1	2	2	4
CAdV-2	---	1	1
CPV-2	4	2	6

Source: Elaborated by the authors.

Coinfections of CDV with other infectious microorganisms have been reported (DAMIÁN et al., 2006; SAITO et al., 2006; CHVALA et al., 2007, HEADLEY et al., 2013), suggesting that the immunosuppressive effects of CDV may facilitate the maintenance of other pathogens (DAMIÁN et al., 2006; CHVALA et. al., 2007; HEADLEY et al., 2013). Saito et al. (2006) described 22 cases of dogs that had clinical signs of canine distemper associated with viruria. According to Negrão, Alfieri and Alfieri (2007), more than one type of clinical sample should be evaluated for CDV considering the different clinical manifestations of canine distemper. Therefore, urine should be included as biological material for the *ante mortem* diagnosis of CDV by RT-PCR since it is easier to collect than cerebrospinal fluid (CSF) and as sensitive as leucocytes, biological samples normally used for diagnosis of CDV.

Urine excretion occurs in CAdV-1 infection as the virus disseminate to several tissues and body secretions after viremia. CAdV-1 persists in the renal tubular epithelium for 14 days after infection (GREENE, 2012), suggesting viruria. Headley et al. (2013) have described the detection of CAdV-2 DNA in urine by PCR, also suggesting excretion via urinary system.

CPV-2 produces lesions predominantly within the gastrointestinal tract (GREENE, DECARO, 2012). After viremia, the virus can be detected in lymphoid tissues, lungs, spleen, liver, myocardium, and kidneys (GREENE, DECARO, 2012). However, the excretion of CPV-2 via the urinary system has not been previously described as far as the authors of this study are aware.

PCR and RT-PCR are helpful diagnostic tools due to their elevated specificity and sensitivity and quick results, assisting the clinician to establish the best possible treatment for the patient. As it has been previously described in CDV studies, urine is a more sensitive sample than serum and leucocytes in RT-PCR assays, and easier to collect than CSF, making urine a good biological sample for the *ante mortem* diagnosis of CDV (SAITO et al., 2006; NEGRÃO; ALFIERI; ALFIERI, 2007). These findings suggest that urine excretion might be an important route for maintaining these viruses in the environment, and should be considered as a source of infection for healthy dogs. The reduced detection of CAdV-1, CAdV-2, and CPV-2 viruria in this study may be due to the lack of medical history and physical examination of the dogs, and to a sampling bias, since the urine samples were sent for the laboratorial diagnosis of canine distemper. It would be worthy to investigate if the CDV, CAdV-1, CAdV-2, and CPV-2 associated viruria has any relationship on the stage of infection and clinical signs presented by the affected dog, and for how long these viruses are excreted via the urinary system after the infected animal has clinically recovered.

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