NOTA PRÉVIA

Canine distemper virus: detection of viral RNA by Nested RT-PCR in dogs with clinical diagnosis

Vírus da cinomose canina: detecção do RNA viral pelo Nested RT-PCR em cães com diagnóstico clínico

Rosana ALCALDE[1,a]; Marcia Mery KOGIKA[2,a]; Vera A. Batistini FORTUNATO[2]; Bruna Maria P. COELHO[2]; Luciano Rodrigo LOPES[3]; Paulo Bandiera PAIVA[3]; Edison Luiz DURIGON[1]

[1]Departamento de Microbiologia do Instituto de Ciências Biomédicas da Universidade de São Paulo, São Paulo, SP, Brazil
[2]Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, SP, Brazil
[3]Departamento de Informática em Saúde da Universidade Federal de São Paulo, São Paulo, SP, Brazil

Abstract

Canine distemper virus (CDV) is a pathogen which affects dogs and causes severe disease leading to death. Dogs infected with CDV can be diagnosed by RNA detection by Nested PCR technique. The following study proposed to evaluate CDV RNA in blood, urine and saliva samples. The Nested-PCR technique was able to detect CDV RNA in different types of biologic samples. The higher number of positive results was obtained in urine samples.

Keywords: Distemper. Nested-PCR. CDV.

Resumo

O vírus da cinomose canina (CDV) é um patógeno que afeta cães, causando doença grave e que pode levar a morte. Os cães infectados pelo CDV podem ser diagnosticados pela detecção do RNA utilizando-se a técnica de Nested-PCR. O presente estudo teve como objetivo avaliar o RNA do CDV no sangue, urina e saliva em cães com diagnóstico clínico de cinomose. A técnica de Nested-PCR foi capaz de detectar o RNA em diferentes tipos de amostras biológicas. Obteve-se um maior número de resultados positivos em amostras de urina.

Palavras-chave: Cinomose. Nested-PCR. CDV.

Canine distemper virus (CDV) is a highly contagious pathogen which may cause lethal systemic disease in dogs and other carnivores throughout the world[1]. Affected dogs usually present gastrointestinal and respiratory clinical signs, the central nervous system (CNS) is frequently affected and the dogs could develop depression, muscular spasms and pelvic limbs paralysis, and progressive loss of weight[2,3,4,5].

The aim of this study was to detect the CDV nucleoprotein (NP) gene in dogs presenting clinical signs of the disease in different clinical stages. Reverse Transcription-PCR (RT-PCR) and Nested-PCR were performed for a rapid NP gene detection in biologic samples such as peripheral blood mononuclear cells (PBMCs), urine, and saliva.

Samples were obtained from 100 dogs (37 female and 63 male) clinically suspected of CDV infection. The dogs showed leucopenia, as well as respiratory, gastrointestinal, and central nervous system implications.

Correspondence to:
Rosana Alcalde
Laboratório de Dermatologia e Imunodeficiências LIM56/FMUSP
Faculdade de Medicina
Universidade de São Paulo
Av. Dr. Enéas de Carvalho Aguiar, 470, IMT II, terceiro andar
Tel.: +55 11 3061 7194; Fax: +55 11 3081 7190
E-mail: rosana_fmusp@yahoo.com.br

Received: 13/06/11
Approved: 06/02/13

[a] Both authors contributed equally for this study

Research supported: Fundação de Amparo a Pesquisa (FAPESP)

All dogs involved in the study were not vaccinated for distemper and had typical clinical sings regarding to sub acute and chronic development of the disease. The dogs were assisted at the Veterinary Teaching Hospital of the School of Veterinary Medicine, Universidade de São Paulo. Their ages ranged from 2 months to 16 years. Fifty-four dogs were mongrel while 46 were classified as specific breeds.

In order to detect the CDV nucleoprotein in different biological samples, PBMCs were obtained by differentials of gradient centrifugation for RNA extraction. Urine samples were centrifuged and sediments were stored with trizol (Invitrogen™ Life Technology, USA) as well as saliva samples were stored with trizol for RNA extraction.

The RT-PCR technique was performed according to Shimizu, Shimizu and Burns[6] To detect the NP gene by both RT-PCR and the Nested-PCR methods, two sets of primers were designed based on the sequence of Onderstpoort strain, GenBank accession number (AF014953). Sequences of primers used in PCR and Nested-PCR were: primers (outer) RMBV1 5'-TAAGCTGGGTCAAAGTAAGATCG-3' (1167 to 1189) and RMBV3 5'-GAATTGCTGAAATGATTTGTGAT-3' (853 to 875); primers (inner) RMBV2 5'-TTGGCATTGAAACTATGTATCC-3' (930 to 952) and RMBV32 5'-CGAAACCCAACCCTCCCATG-3' (1145 to 1164). The expected product was 336 and 234-base pair (bp), respectively.

According to the NP gene Nested-PCR standardization, one product with 336bp and one product with 234bp were obtained. The Onderstpoort vaccine strain was used, as a positive control.

Sixty-one infected dogs (61%) had at least one sample (PBMCs, urine and/or saliva) detected by Nested-PCR. Thirty-eight PBMC samples and 44 urine samples were detected as positive, while only 12 saliva samples were positive by Nested-PCR. However, the single-step RT-PCR technique could only detect 6% as positive (urine sample = 4; PBMC sample = 1; saliva sample = 1). Therefore, the results obtained by those two methods were different (Fisher's Exact Test $p < 0.005$) in urine (sensitivity = 8.3%; specificity = 36.7%; positive predictive value = 4%; negative predictive value = 56%; likelihood ratio = 0.132), PBMC (sensitivity = 2.5%; specificity = 38.5%; positive predictive value = 1%; negative predictive value = 62%; likelihood ratio = 0.042) and saliva (sensitivity = 7.7%; specificity = 47%; positive predictive value = 1%; negative predictive value = 88%; likelihood ratio = 0.145) samples.

Thus, this study showed that Nested-PCR method was more effective to detect NP gene compared to RT-PCR; in urine samples, the detection of NP gene had higher frequency of positivity (44%), followed by PBMCs (38%) and saliva (12%). In other biological samples, CDV NP gene could also be detected as it was observed by Elia et al.[7] in swabs using the RT-PCR test. In agreement with the findings of the present study, Gebara et al.[8] demonstrated that regardless of the clinical stage of the illness, urine samples was more adequate for the detection of CDV, however the method used was RT-PCR. Similar results were also observed by Saito et al.[9] in the *pre-mortem* diagnosis of distemper in dogs. Although RT-PCR method has been applied successfully to the diagnosis of CDV[7], in the present study, Nested-PCR technique showed more effectiveness for the diagnosis of CDV infection[10].

In summary, comparing RT-PRC and Nested-PCR methods for the identification of NP mRNA of CDV, Nested-PCR was successfully achieved through the use of primer sets designed specifically for this test in the diagnosis of infection for the pre-mortem diagnosis, in sub-acute or chronic clinical presentations of the disease, in different biological samples (urine and PBMC, mainly), and then further studies should be conducted in order to contribute for CDV methods of diagnosis.

Acknowledgments

The authors thank all the patients who participated in this study. The authors thank Luis Carlos Vieira and Dr Mitika Kuribayashi Hagiwara for technical assistance and, for the correction of this work, Dr Jorge Casseb, Dr Shirley Vasconcelos Komninakis, Mr Brent Hester and Fapesp.

References

1. MARTINEZ, J. S.; ARVIZU, R. U.; SORIANO, V. E.; FAJARDO, R. Identification of a genetic variant of the canine distemper virus from clinical cases involving two vaccinated dogs in Mexico. Veterinary Journal, v. 175, n. 3, p. 423-426, 2008.
2. APPEL, M. J. G.; SUMMERS, B. A. Pathogenicity of morbilliviruses for terrestrial carnivores. **Veterinary Microbiology**, v. 44, n. 2-4, p. 187-191, 1995.
3. KEAWCHAROEN, J.; THEAMBOONLERS, A.; JANTARADSAMEE, P.; RUNGSIPIPAT, A.; POOVORAWAN, Y.; ORAVEERAKUL, K. Nucleotide sequence analysis of the nucleocapsid protein gene of canine distemper virus isolates in Thailand. **Veterinary Microbiology**, v. 105, n. 2, p. 137-142, 2005.
4. KIM, D.; JEOUNG, S. Y.; AHN, S. J.; LEE, J. H.; PAK, S. I.; KWON, H. M. Comparison of tissue and fluid samples for the early detection of canine distemper virus in experimentally infected dogs. Journal Veterinary Medical Science, v. 68, n. 8, p. 877-879, 2006.
5. AMUDE, A. M.; ALFIERI, A. A.; ALFIERI, A. F. Clinical pathological findings in dogs with distemper encephalomyelitis presented without characteristic signs of the disease. **Research in Veterinary Science**, v. 82, n. 3, p. 416-422, 2007.
6. SHIMIZU, H.; SHIMIZU, C.; BURNS, J. C. Detection of novel RNA viruses: morbilliviruses as a model system. **Molecular and Cellular Probes**, v. 8, n. 3, p. 209-214, 1994.
7. ELIA, G.; DECARO, N.; MARTELLA, V.; CIRONE, F.; LUCENTE, M. S.; LORUSSO, E.; TRANI, L. D.; BUONAVOGLIA, C. Detection of canine distemper virus in dogs by real-time RT-PCR. **Journal Virology Methods**, v. 136, n. 1-2, p. 171-176, 2006.
8. GEBARA, C. M. S.; WOSIACKI, S. R.; NEGRÃO, F. J.; OLIVEIRA, D. B.; BELONI, S. N. E.; ALFIERI, A. A.; ALFIERI, A. F. Detection of the canine distemper virus, nucleoprotein gene by RT-PCR in urine of dogs with distemper clinical signs. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 56, n. 4, p. 480-487, 2004.
9. SAITO, T. B.; ALFIERI, A. A.; WOSIACKI, S. R.; NEGRÃO, F. J.; MORAIS, H. A. S.; ALFIERI, A. F. Detection of canine distemper virus by reverse transcriptase-polymerase chain reaction in the urine of dogs with clinical signs of distemper encephalitis. **Research in Veterinary Science**, v. 80, n. 1, p. 116-119, 2006.
10. KIM, Y. H.; CHO, K. W.; YOUN, H. Y.; YOO, H. S.; HANN, H. R. Detection of the canine distemper virus (CDV) through one step RT-PCR combined with nested PCR. **Journal Veterinary Science**, v. 2, n. 1, p. 59-63, 2001.