(REVIEW ARTICLE)

# Primers design for the detection and genotyping of the *Canine Distemper Virus*

Garrido, C., Jara, MA., and Navarro, C. *

*Department of Animal Medicine Preventive, Faculty of Veterinary Sciences, University of Chile, Santiago, Chile.*

## Abstract

*Canine Distemper Virus* (CDV) has potential hosts, all carnivores, including wild animals. The associated disease, called canine distemper (CD) has a high prevalence and is highly contagious, being one of the main causes of death in domestic and wild canids. Although it is a monotypic virus, that is, with little genetic preference, the H gene has a high genetic variation, which would produce different strains of the virus, some more virulent than others and with different tropism. It is because of this specific specification that patients still get sick, despite being vaccinated, since the eightfold vaccine protects against one strain of the virus: America-1.

Thus, any carnivore susceptible to the virus that is confronted with a strain against which it has not been vaccinated is as likely to become ill as an animal that has simply never been vaccinated against this viral disease. To face this situation, it is essential to know the characteristics of the genotypes of the virus present in the country. Through previous studies, it is known that in Chile there are at least two of the fourteen VDC lineages, but this does not imply that they are the only ones in the country, which is why it is proposed to propose a prior Polymerase Chain Reaction protocol. Reverse transcription using both matches for the N gene and for the H gene (lineages: America-1 and European) to shed light on the genotype found in the samples, which may not even belong to either of the two lineages described above in the country.

For the generation of an RT-PCR protocol capable of detecting VDC, the use of the Genbank® genomic database in conjunction with the *in silico* design of primers is very interesting, indicating whether it corresponds to the American-1 or European lineage.

**Keywords:** Canine Distemper; Detection; Extinction animals; Virus

## 1. Introduction

Canine distemper virus (CDV) is a lipid-enveloped pleomorphic virus belonging to the genus Morbillivirus of the *Paramyxoviridae* family, *Mononegavirales* order and is antigenically related to rinderpest and measles viruses. As the name of its order indicates, it is a negative-sense single-stranded RNA virus with a helical nucleocapsid. Its unsegmented genome is about 15.7 kilobases (kb) [1]. The viral genome encodes for six structural proteins: the nucleocapsid protein (N), the phosphoprotein (P), the matrix protein (M), the polymerase (L), and the envelope glycoproteins: the fusion protein (F) and hemagglutinin (H). The N, P and L proteins together with the viral RNA form the ribonucleoprotein complex (RNP), which directs the sequential synthesis of mRNA from viral genes and the replication of antigenomes. The F and H proteins are integral proteins of the lipid envelope membrane, associated with the M protein, which carry out the interaction between the RNP complex and the membrane. In addition to this, integral membrane proteins are responsible for virion recognition for entry into host cells, being the main targets of the immune system [2, 3]. In vitro studies have also shown that hemagglutinin, the protein, is the main determinant of cell tropism [4].

*Corresponding author: canavarr@uchile.cl
Department of Animal Medicine Preventive, Faculty of Veterinary Sciences, University of Chile, Santiago, Chile.

It is an enveloped virus, so it is very sensitive to the environment, however, its constant elimination through reactions and body fluids, from the seventh day after infection and its high infectivity, allow its rapid spread in the ecosystem. . In addition to this, there are animals that excrete the virus before showing signs [4].

## 1.1. Host Range

The VDC has numerous hosts, all of them belonging to the order of carnivores such as *Canidae* (dogs, foxes, wolves, dingoes, and coyotes), *Procionidae* (raccoons, coatis), *Mustelidae* (ferrets and mink), *Felidae* (tigers, lions, leopards) and even in marine mammals [4, 5, 6]. On the other hand, a possible relationship between Paget's disease in humans and CDV infection has been demonstrated, due to the detection of viral RNA in affected tissues [7, 8]. In Chile, in 1994, the first isolation of the virus in cell cultures was carried out, it was reported inoculated with reactions from a canine with clinical signs of CD, where the clinical diagnosis was achieved by electron microscopy and histopathological studies [9]. In 2003, an outbreak of CD occurred in the endemic populations of foxes in the Fray Jorge National Park (Coquimbo Region) and it was speculated that it could be related to the existence in native mustelids such as chingue and quique [10]. Four years later, in 2007, an outbreak of DC occurred on Robinson Crusoe Island (Valparaíso Region), where several domestic dogs were affected, but not the island's endemic sea lions [11].

## 1.2. Pathogeny

The course of the disease is variable and largely depends on the efficacy of the host's immune response. Those affected are puppies between three and six months of age, since their immune system is poorly developed and they have already lost the main maternal results [4, 6]. The virus enters the respiratory tract and during the first 24 hours it affects the regional lymph nodes and after 7 days all the lymphoid tissue, producing immunosuppression due to the decrease in the appearance of T and B lymphocytes [3]. In the following weeks, the animal may recover or present symptoms associated with a secondary viremia, since the infected mononuclear cells transport the virus to the epithelial surface of the digestive, respiratory, urogenital, and cutaneous and/or central nervous system tracts, with the respective clinical manifestations of each affected organ [5]. Currently, in addition to antibiotic treatment to prevent secondary infections, which are very common in immunosuppressed animals, there is no effective treatment against CDV (6). Most of the vaccines used correspond to polyvalent attenuated virus vaccines, which provided limited protection and also have the risk of causing diseases, because they maintain their lymphotropism and ability to induce immunosuppression [6, 12]. Recombinant vaccines are a safer option as they spare the pathogen and use some of its boosters to stimulate a host immune response. These vaccines show high efficacy, with higher affinity and duration of test production than attenuated virus vaccines [13].

## 1.3. Diagnostic methods

There is a wide variety of laboratory diagnoses that can be performed to differentiate other diseases with similar symptoms. Among them are:

- ELISA for the detection of specific IgM against VDC: This test is quite useful, since immunoglobulin M in dogs terminated by VDC persists for 5 weeks to 3 months depending on the strain and the immune response of the recipient. Sampling should be done at least 3 weeks after vaccination against the virus, since IgM persists around this time in the animal, which can result in a false positive [14].
- Molecular techniques (PCR): The polymerase chain reaction associated with the reverse transcription of the viral genome (RT-PCR) is used for the epidemiology of the virus and the dynamic circulation of the different existing strains. Using this technique and based on the sequencing of the H gene, it has been possible to determine the existence of at least 14 different strains of the virus: Europe-1/South America-1, South America-2, South America-3, and Europe wild, Asia-4, America-2, Rockborn-Like, Africa-2, Asia-1, Arctic, Africa-1, Asia-2, Asia-3, and America-1 [12]. Of these lineages, it has been proven that in Chile, there are at least two of them, the America 1 and Europe-1/South America-1 lineage [15].

Thus, this performance proposes the use of a genomic database in conjunction with the *in silico* design of primers multiplex RT-PCR protocol for detection and genotyping of Canine Distemper Virus in Chile, by obtain specific primers for the detection of both the VDC N gene and the H gene (America-1 and European-1 genotypes) of VDC and propose the conditions for the development of a multiplex RT-PCR protocol.

## 2. Material and methods

The present work will be carried out in the any basic laboratory of Biology, Biochemistry or Virology around the world.

## 2.1. Information available

In the first instance, the GenBank ® accession numbers for the CDV H gene sequences will be used, in order to obtain the nucleotide sequences for the N gene (complete genome, complete N gene) and for the H gene (complete lineage). America-1 and European-1 lineages), available in a phylogenetic tree [16].

### 2.1.1. Design of primers from candidate nucleotide regions.

All VDC N gene sequences described in Genbank® and all H gene sequences (America-1 and Europe-1 genotypes) used in the construction of a VDC phylogenetic tree will be considered [16].

The sequences obtained for each gene will be aligned using the Clustal Omega program, which will allow the construction of the consensus nucleotide sequence in each case.

Then, this consensus sequence will be entered into Invitrogen's Oligo Perfect Design ® program or another similar one, in order to know the nucleotide sequence of the best pair of proposed matches.

### 2.1.2. Proposal of the conditions for the use of the conventional PCR technique

The generation of primers indicated involves the determination of the optimum alignment temperature, key in the PCR technique. This temperature is proposed by the indicated program when delivering the Tm (melting temperature) of each proposed splitter. Thus, the final proposal for conventional PCR conditions or protocol will consider the typical ones described [17] that involve at least 30 cycles, with a denaturation step at 94-95°C for 1 minute, followed by of the alignment step at the temperature suggested by the Tm of the proposed matches and finally an extension step at 72°C. A final step of 10 minutes at 72°C will be suggested as well as the use of a temperature gradient thermocycler for the optimal selection of the alignment temperature.

## 3. Discussion

This text has described one of the ways of detecting and genotyping canine distemper virus, taking as an example the current situation in Chile regarding the pathogen that causes the disease. Obviously, the idea can be applied to any geographical reality and especially to animals in danger of extinction. If we can, you can too!

## 4. Conclusion

The fantastic idea of Kary Mullis in conjunction with the design of primers can make the difference between updated diagnostic methods. Not only in the field of virology.

## Compliance with ethical standards

### Acknowledgments

### Disclosure of conflict of interest

The authors have not declared conflict of interest.

## References

[1]     Lamb, R; Parks, G. 2007. *Paramyxoviridae*: The viruses and their replication. Fields Virology.

[2]     Summers, B; Appel, M. 1994. Aspects of *Canine Distemper Virus* and measles virus encephalomyelitis. Neuropathology and Applied Neurobiology 20: 525-534.

[3]     Von Messling; V. Zimmer, G. Herrler, G; Haas, L; Cattaneo, R. 2001. The hemagglutinin of canine Distemper virus determines tropism and cytopathogenicity. Jounnal of Virology 75: 6418-6427.

[4] Stern, L; Greenberg, M; Gershoni, J; Rozenblatt, S. 1995. The hemagglutinin envelope protein of canine distemper virus (VDC) confers cell tropism as illustrated by VDC and measles virus complementation analysis. J Virol 69: 1661-1668.

[5] Appel, M; Summers, J. 1999. Distemper canino: Estado actual. Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA. p. 5.

[6] Martella, V; Elia, G; Buonavoglia, C. 2008. Canine distemper virus. Veterinary Clinics of North America Small Animal Practice 38: 787-797

[7] Frisk, A; König, M; Moritz, A; Baumgärtner, W. 1999. Detection of canine Distemper virus nucleoprotein ARN by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with Distemper. JouARNl of Clinical Microbiology 37: 3634-3643.

[8] Mee, A; Dixon, J; Hoyland, J; Davies, M; Selby, P. 1998. Detection of canine Distemper virus in 100% of Paget's disease samples by in situ-reverse transcriptasepolymerase chain reaction. Bone 23: 171-175.

[9] Cerda, L; Mathieu, C; Quinteros, G. 1994. Primer aislamiento de Virus Distemper Canino en Chile. XIV Congreso Panamericano de Ciencias Veterinarias, Acapulco, México.

[10] Moreira, R; Stutzin, M. 2005. Study of the mortality of foxes in the IV Region. Official Veterinary Bulletin. SAG, Chile 3: 1-8.

[11] Jara, C; Matus, P; Moreira, R. 2007. Canine distemper on Robinson Crusoe Island (Juan Fernández Archipielago, V Region): Antecedents of an epidemic outbreak. Official Veterinary Bulletin. SAG, Chile. 8: 1-19.

[12] Keawcharoen, J; Theamboonlersc, A; Jantaradsameec, P; Rungsipipatb, A; Poovorawanc, Y. 2005. Nucleotide sequence analysis of nucleocapsid protein gene of canine Distemper virus isolates in Thailand. Veterinary Microbiology 105: 137-142.

[13] Larson, L; Schultz, R. 2006. Effect of vaccination with recombinant canine Distemper virus vaccine immediately before exposure under shelter-like conditions. Vet Ther 7: 113-118.

[14] Appel, M; Summers, B. 1995. Pathogenicity of morbilliviruses for terrestrial carnivores. Vet Microbiol 44: 187-191.

[15] Salas, V; Pizarro, J; Navarro C. 2018. Phylogenetic analysis of canine distemper virus detected in Chile. IJCR 10: 72402-72407.

[16] Ke, G.; Ho, C.; Chiang, M.; Sanno-Duanda, B.; Chung, C.; Lin, M.; Shi, Y.; Yang, M.; Tyan, Y.; Liao, P.; Chu, P. 2015. Phylodynamic analysis of the canine distemper virus hemagglutinin gene. [en línea] BMC Vet. Res. 11:164.

[17] Mullis, K; Faloona, F. 1987.Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods in Enzymology, 155:335-350.