



First Genome Sequence of a Canine Distemper Virus Strain from South America

Nicolás Sarute,^a María V. Delgado,^a Lucía Carrau,^a Alejandro Benech,^b Lourdes Francia,^a Ruben Pérez,^a Yanina Panzera^a

Sección Genética Evolutiva, Instituto de Biología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay^a; Departamento de pequeños animales, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay^b

Canine distemper virus causes a severe infectious disease in carnivores worldwide. Herein, we sequenced and analyzed the genome of a new strain (Uy251/2012) isolated from a dog in Uruguay. The Uy251/2012 strain belongs to the Europe1/South Americal lineage, and constitutes the first report of a genomic sequence in South America.

Received 28 August 2014 Accepted 10 September 2014 Published 23 October 2014

Citation Sarute N, Delgado MV, Carrau L, Benech A, Francia L, Pérez R, Panzera Y. 2014. First genome sequence of a canine distemper virus strain from South America. Genome Announc. 2(5):e01009-14. doi:10.1128/genomeA.01009-14.

Copyright © 2014 Sarute et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Yanina Panzera, ypanzera@fcien.edu.uy.

Canine distemper virus (CDV) belongs to the *Morbillivirus* genus, *Paramyxoviridae* family, and possesses a nonsegmented single-stranded negative RNA genome that encodes six structural proteins (1). CDV is the etiological agent of distemper, a severe systemic disease that affects domestic and wild carnivores worldwide. The disease is characterized by several clinical signs (respiratory, gastrointestinal, and/or central nervous system) and high mortality rates (2).

For decades, the disease was controlled using attenuated vaccines; however, CDV outbreaks are constantly reported in dogs and wildlife, with an increase in the number of infected dogs, both vaccinated and unvaccinated (3).

CDV strains are classified into geographic lineages based on the variability of the hemagglutinin (H) and the fusion protein signal-peptide (Fsp) (4, 5).

Herein, we amplified the complete coding and intergenic regions of the Uy251/2012 South American strain obtained from a dog's necropsy. The viral RNA was isolated from a lung slice (~150 mg) using Trizol reagent (Invitrogen) according to the manufacturer's instructions; the first-strand cDNA was synthesized using random primers (Invitrogen). Nine pairs of primers were designed based on the reference sequence CDV 5804 strain (AY386315). Phusion High-Fidelity DNA Polymerase (Thermo Scientific) was used to amplify the genome in nine overlapping fragments. Purified amplicons were sequenced bidirectionally using an ABI3130 genetic analyzer (Applied Biosystems). Sequences were compiled and edited using the SeqMan program (Lasergene). The coding genome of the Uy251/2012 strain consists of 15,447 bp and encompassed six genes (3' N-P-M-F-H-L 5'). The Uy251/2012 strain genome was compared with 40 complete genomes available at the Genbank database belonging to six CDV lineages previously described. Based on the phylogenetic analysis (maximum likelihood-general time reversible model, Gamma distribution), the Uy251/2012 strain was grouped within the Europe1/South America1 lineage (EU1/SA1), as reported for all the Uruguayan strains previously analyzed (5-7). The nucleotide and amino acid (nt/aa) identity within this lineage was 98.2/98.8%,

respectively. The mean nt/aa identities between the EU1/SA1 and other lineages were 95.9/96.3% for North America1, 94.8/95% for Asia1, 94.5/94.9% for Asia2, 94.9/93.2% for North America2, and 94.2/94.2% for Europe3. The comparison with the vaccine strains showed lower values of nt/aa identity of 92.5/92.6%, respectively.

When comparing each individual gene the following values for nt/aa identities were obtained: 95.7/97.5% (N gene), 95.6/94.3% (P gene), 95.5/98.2% (M gene), 92.8/91.7% (F gene), 92.4/92.1% (H gene), and 94.6/85.3% (L gene). These values are in agreement with previous studies that established that the H and F genes are highly variable (4, 8, 9); strikingly, the highest amino acid divergence was detected for the L protein, which has not been analyzed for CDV field strains to date.

The Uy251/2012 strain genome sequence is the first report for South America so far. The high genetic variability between Uy251/ 2012 and vaccine strains should be considering for the formulation of new and updated vaccines for South America. Further analysis, including more genomic sequences, will contribute to the knowledge of the CDV evolutionary landscape in this region.

Nucleotide sequence accession number. This sequence was deposited at GenBank under the accession number KM280689.

ACKNOWLEDGMENTS

This study was supported partially by Programa de Desarrollo de las Ciencias Básicas (PEDECIBA) and Comisión Sectorial de Investigación Científica (CSIC).

REFERENCES

- 1. Lamb RA, Parks GD. 2007. Paramyxoviridae: the viruses and their replication, p 1449–1496. *In* Fields BN, Knipe DV, Howley PM (ed), Fields virology, 5th ed. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia.
- 2. Appel MJG. 1987. Canine distemper virus, p 133–159. *In* Virus infections of carnivores, 1st ed, vol 1. Elsevier Science, Amsterdam.
- 3. Demeter Z, Palade EA, Hornyák A, Rusvai M. 2010. Controversial results of the genetic analysis of a canine distemper vaccine strain. Vet. Microbiol. 142:420–426. http://dx.doi.org/10.1016/j.vetmic.2009.10.017.
- Martella V, Cirone F, Elia G, Lorusso E, Decaro N, Campolo M, Desario C, Lucente MS, Bellacicco AL, Blixenkrone-Møller M, Carmichael

LE, Buonavoglia C. 2006. Heterogeneity within the hemagglutinin genes of canine distemper virus (CDV) strains detected in Italy. Vet. Microbiol. 116:301–309. http://dx.doi.org/10.1016/j.vetmic.2006.04.019.

- Sarute N, Calderón MG, Pérez R, La Torre J, Hernández M, Francia L, Panzera Y. 2013. The fusion protein signal-peptide-coding region of canine distemper virus: A useful tool for phylogenetic reconstruction and lineage identification. PLoS One 8:e63595. http://dx.doi.org/10.1371/ journal.pone.0063595.
- Panzera Y, Calderón MG, Sarute N, Guasco S, Cardeillac A, Bonilla B, Hernández M, Francia L, Bedó G, La Torre J, Pérez R. 2012. Evidence of two co-circulating genetic lineages of canine distemper virus in South America. Virus Res. 163:401–404. http://dx.doi.org/10.1016/ j.virusres.2011.10.008.
- Sarute N, Pérez R, Aldaz J, Alfieri AA, Alfieri AF, Name D, Llanes J, Hernández M, Francia L, Panzera Y. 2014. Molecular typing of canine distemper virus strains reveals the presence of a new genetic variant in South America. Virus Genes 48:474–478. http://dx.doi.org/10.1007/ s11262-014-1054-z.
- Bolt G, Jensen TD, Gottschalck E, Arctander P, Appel MJ, Buckland R, Blixenkrone-Møller M. 1997. Genetic diversity of the attachment (H) protein gene of current field isolates of canine distemper virus. J. Gen. Virol. 78:367–372.
- Pardo ID, Johnson GC, Kleiboeker SB. 2005. Phylogenetic characterization of canine distemper viruses detected in naturally infected dogs in North America. J. Clin. Microbiol. 43:5009–5017. http://dx.doi.org/ 10.1128/JCM.43.10.5009-5017.2005.