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Research Article

How to Identify Possible New Genotypes of Canine Distemper Virus?

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Summary

Canine Distemper Virus (CDV) is the causal agent of a complex infection present in a wide range of hosts. It is a viral pathogen that affects various species orders, including several families of carnivores, primates and even cetaceans. CDV is lymphotropic, neurotropic and epitheliotropic, which causes a multisystemic infection with diverse clinical signs. It generally affects young carnivores and is characterized by signs such as: anorexia, mucopurulent conjunctivitis, nasal discharge, dyspnea, and even pneumonia. Mortality can be high in previously unexposed animals. This virus can be transmitted to wildlife, including aquatic carnivores, through contact with domestic canids. This transmission route is increasingly common, because domestic species usually invade the habitat of wild species, which facilitates contact. The clinical diagnosis of the disease is complex due to the wide range of signs present, which makes confirmation by means of laboratory tests necessary. Thus, the reference test is the Reverse Transcription Polymerase Chain Reaction (RT-PCR), which shows the presence of a conserved gene and with which the viral strains have also been characterized based on the analysis of the H gene, which is the most variable. Using the RT-PCR technique, the presence of several circulating VDC genotypes in various hosts around the world has been determined, of which two have been described in Chile, these being the America-1 and Europe-1/South America-1 genotypes. In this work, a phylogenetic tree will be constructed regarding the H gene of VDC using the Genbank® database, and conclusions can be obtained by comparing it with previous data from the literature.

Background

Etiological Agent

Canine Distemper (CD), also known by the alternative names of "canine distemper", "hard pad" or Carré's disease, is a worldwide disease caused by the Canine Distemper Virus (CDV), a viral species belonging to the *Mononegavirales* order, *Paramyxoviridae family*, *Morbillivirus* genus [1]. CDV mainly affects canids and other families of carnivores, such as felines, viverrids (civets, genets), mustelids (otters, ferrets) and procyonids (raccoons, coatis). However, the hosts of CDV have expanded in recent years, and cases related to this agent have been observed in other species, such as pinnipeds and phocids [2,3].

Virion Genome and Structure

The VDC has a single-stranded, negative-sense, non-segmented RNA genome of about 15,700 nucleotides in length. It contains six genes that encode the virion proteins *Murphy et al*., 1999 [4]. The nucleocapsid, encoded by the N gene (1.5 kb), is the protein responsible for protecting the viral RNA. The matrix protein, encoded by the M gene (1 kb), plays important roles in virion morphology and assembly. The Large polymerase, encoded by the L gene (6.5 kb), and the phosphoprotein, encoded by the P gene (1.5 kb), are the proteins that form a functional polymerase complex and are responsible for viral RNA replication *Murphy et al.,* 1999a; [4]. The fusion protein, encoded by the F gene (1.9 kb), is an essential glycoprotein for mediating the fusion between the viral particle and the host cell membrane, providing the mechanism necessary for passing from one cell to another. The hemagglutinin, encoded by the H gene (1.8 kb), is the glycoprotein responsible for viral adhesion to the host cell *Murphy et al.,* 1999a; [4]. Finally, the P gene (1.5 kb) is highly conserved and, as in other viruses belonging to the *Paramyxoviridae* family, is polycistronic and encodes three distinct proteins: P, V and C [5,6,7,8]. The P protein is essential for viral replication, is synthesized in excess in infected cells, and shows a high turnover rate; presenting transitory functions during the assembly of the

nucleocapsid and the synthesis of RNA. On the other hand, the C and V proteins are considered non-essential for replication. The P protein bound to the L protein integrates the polymerase complex responsible for RNA synthesis (transcription and replication) [7,9].

Pathogenesis and Pathology

The pantropic nature of the virus is expressed by affecting various tissues of the host, to such an extent that it is considered as the first pre-diagnosis in susceptible canids with multisystemic disease. The latter is due to the fact that VDC is transported by macrophages and other cells of the immune system, via the blood (viremia) and/or lymphatic system. Among the main signs that can be observed, it is possible to mention: cough, rhinitis/serous or mucopurulent conjunctivitis due to secondary bacterial contamination, diarrhea, vomiting, convulsions, cerebellar syndrome (mobility and coordination problems), paralysis and muscle spasms of nervous origin, among others [10].

Diagnosis

(clinical, serological, viral isolation and molecular techniques). The diversity of signs makes diagnostic research difficult and some techniques such as immunohistochemistry and ELISA do not satisfy in terms of sensitivity, specificity and speed [11]. Molecular methods based on the Polymerase Chain Reaction (PCR) preceded by Retrotranscription (RT) of genomic RNA provide a diagnosis of high sensitivity, high specificity and rapidity [3].

Polymerase Chain Reaction (PCR)

The PCR technique has been successfully applied for the detection of viral nucleic acid, as it is a highly specific, rapid and sensitive method for the antemortem diagnosis of CDV infection in dogs, regardless of the presentation of CDV, the humoral immune response and the distribution of the viral antigen [11-13]. Generally, for the detection of CDV by RT-PCR, conserved regions within the viral genome, such as the nucleocapsid protein gene (N gene) are used as the main targets for amplification [11,13]. The RT-PCR technique has allowed the study of viral genetic variations, through the molecular characterization of the H gene sequence of the genome, currently describing at least 14 circulating lineages of the VDC: Africa-1; Africa-2; America-1; America-2; Asia-1; Asia-2; Asia-3; Asia-4; Europe-1/South America-1; Europe-2 (Wild European); Europe-3 (Arctic); Rockborn-like; South America-2; South America-3 [14].

Genetic Characterization in South America

The first report of characterization of the VDC strains circulating in South America was registered in Argentina *Calderón et al.,* 2007. This study showed the co-circulation in that country of two genetic variants or genotypes [15]. *Calderón et al.* (2007) analyzed H gene fragments by restriction fragment length polymorphism (RFLP) and sequencing. A single Nde I site (restriction site) was detected in all 24 wild-type strains, but was absent in the vaccine strains *Calderón et al.,* 2007; [16].

In the State of Paraná in Brazil, it was determined that circulating CDV strains are closely related to those of the Europa 1 lineage of CDV, with marked differences from other recognized geographic groups of CDV isolates and from the vaccine strains [17]. Since the Europa 1 lineage was the most prevalent in South America, it was suggested to change the name to Europa 1/South America 1 lineage. The South America 2 lineage was found only in Argentina and was related to wild strains of CDV [18]. In Ecuador, a new CDV lineage was described based on the analysis of the Fsp region of South American strains, initially named South America-3 (SA3) [19]. This lineage was proposed to be named NA4/SA3, due to the results obtained in the study by *Fuques*, 2017, which groups strains NA4 and SA3 within the same clade [16]. In Colombia, studies have described a distinct monophyletic group, clearly separated from the previously identified wild-type and vaccine lineages, which was proposed to be named "South America-3" *Espinal et al.,* 2014. It was later renamed by *Panzera et al.* 2015 as South America 4 (SA4) [20]. In Chile, at least two lineages have been described, corresponding to the America-1 and Europe-1/South America-1 lineages [21]. Finally, in Peru, the existence of a new lineage in South America was reported, which includes strains from Peru, Ecuador and North America called NA4/SA3 [16].

Evolutionary History

The first complete sequence of the VDC H gene dates back to 1975 in the United States and today there are more than 400 sequences in the GenBank database [16]. In 2015, Panzera and collaborators performed an analysis using 208 H gene sequences from 16 countries from samples collected between 1975 and 2011 and estimated that the current circulating strains most likely originate in North America. This ancestor diversified into two ancestral lineages, one of which remained in North America (currently called North America-1), while the second expanded, giving rise to the rest of the lineages that currently circulate worldwide [22,16]. Subsequent phylodynamic analyses support these results, contributing to the knowledge of the evolution and spatiotemporal distribution of the virus [16,22,23].

Phylogenetic Tree and Use of MEGA Software

Viral genetic variability as well as evolution over time is valuable information for, for example, the development of effective long-term vaccines and the monitoring of their effectiveness in the future [24]. Phylogenetic trees are used to visualize the evolutionary relationships between species. The high availability of genetic material information allows the construction of phylogenetic trees in thousands of species and has had important contributions in taxonomy, epidemiology or virology [25]. The MEGA (Molecular Evolutionary Genetic Analysis) software allows estimating evolutionary distances, reconstructing phylogenetic trees, and calculating basic statistical quantities from molecular data [26]. MEGA version 11 adds many methods and tools to keep pace with the growing needs of researchers [27]. This work proposes the development of a phylogenetic tree of CDV to determine CDV genotypes based on data stored until 2024 in Genbank®, which would allow comparisons with existing literature. Thus, the construction of a phylogenetic tree that visualizes the existing VDC genotypes according to data provided by the Genbank® database and establish comparisons with the existing scientific literature.

Materials and Methods

This work can be carried out in any virology laboratory with basic equipment, such as a computer with Internet access. In this sense, the idea, while important, would constitute an update for a model that includes animal viruses and that can be extrapolated to viruses that affect humans.

How Construct a Phylogenetic Tree?

a. The nucleotide sequences of the H gene previously described in the literature should be used to verify the existence of the genotypes described by the authors: Africa-1; Africa-2; America-1; America-2; Asia-1; Asia-2; Asia-3; Asia-4; Europe-1/South America-1; Europe-2 (wild European); Europe-3 (Arctic); Rockborn-like; South America-2; South America-3. These sequences are published in Genbank. GenBank® is the National Institutes of Health (NIH) genetic sequence database, a collection of all publicly available DNA sequences. These sequences will be collected in a Word file, in FASTA format, based on text to represent nucleotide sequences [28].

b. All the H gene sequences existing in Genbank® after the last publication on the subject will be used, with the intention of corroborating the existence of any new genotype of the canine distemper virus. The final search date will be considered as the approval date of this Draft Report.

c. The alignment will be carried out directly with the MEGA program.

How Establish Differences and/or Similarities Between the Phylogenetic Tree Obtained and What was Previously Reported in the Scientific Literature?

The generation of a phylogenetic tree updated to 2024 would allow establishing differences and/or similarities with what was previously reported in the literature, considering for example the work of [23] or that of [16] in reference fundamentally to the number of existing genotypes.

Equipment used: For analysis using the MEGA program, the use of a mobile computer, DELL i5, 2.4 Mhz, 500Mb is proposed.

Results

The results will allow us to compare the phylogenetic tree previous described [16,23] with the current data (2024). This comparison will establish the presence of new CDV genotypes on our planet or their absence.

The above is an appendix to be used when answering the question: Why do animals vaccinated against CDV get sick and die? The answer obviously includes the different genotypes that exist today and that have probably increased but have not been reported or described yet.

Conclusion

In any third world laboratory it is possible to detect, understand and determine the genomic sequences of one or more pathogens that affect humans and/or animals. Kary Mullis' fantastic idea, together with the program that allows us to know the sequence of these fragments, favors the advancement of molecular medicine. Thus, the question posed above can be supplemented.

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Conflict of Interest

None.

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