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Paidimuddala Charitha
M.V.Sc., Student, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Thrissur, India

Ambily R
Assistant Professor, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Thrissur, India

Surya Sankar
Assistant Professor, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Thrissur, India

Sudheesh S Nair
Assistant Professor, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Thrissur, India

Priya PM
Professor and Head, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Thrissur, India

Corresponding Author:
Paidimuddala Charitha
M.V.Sc Student, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Molecular detection of canine distemper virus in dogs by reverse transcription- polymerase chain reaction

Paidimuddala Charitha, Ambily R, Surya Sankar, Sudheesh S Nair and Priya PM

Abstract
Canine distemper (CD) is a highly infectious disease affecting dogs world-wide. It is associated with substantial mortality and morbidity among dogs. The present study involves detection of canine distemper virus (CDV) by reverse transcription - polymerase chain reaction (RT-PCR). A total of 15 deep nasal swabs were collected from dogs with respiratory signs brought to University Veterinary Hospital, Mannuthy. Extraction of RNA followed by RT-PCR was done employing primers targeting *N* gene. Multi-component vaccine was used as positive control. Amplicons of approximately 287 bp specific to CDV were obtained in 4 (26.6 %) samples. Representative positive sample was sequenced using Sanger's dideoxy chain termination method. Phylogenetic tree was constructed using MEGA 11 software.

Keywords: Canine distemper virus, RT-PCR, *N* gene, RNA

1. Introduction
Canine distemper (CD) is a fatal disease affecting dogs all over the world. The causative agent is canine distemper virus (CDV) belonging to genus Morbillivirus of the family *Paramyxoviridae*. The clinical signs of CD vary depending on the age and immune status of the host as well as the virulence of the virus strain and the environmental conditions (Greene and Appel, 1990) [2]. Despite routine vaccination for many decades, CD remains a significant problem in dogs. Because of high titers of antibodies to CDV as a result of previous vaccinations or subclinical/clinical infections, serological methods have little diagnostic value (Shin *et al*., 1995) [5]. Considering the infectious nature of CD and high fatality rates, it is important to expedite the diagnosis procedures in order to confirm the infection and adopt control strategies. Detecting the virus early in infection requires a sensitive, precise and quick approach.
To give appropriate medical treatment that saves the life, particularly when clinical signs are evident, a simple test that can identify CDV quickly and correctly must be accessible. Such a test would also aid in excluding other illnesses. At present, CD can be diagnosed by virus isolation, immunofluorescence assays (IFA), RT-PCR, and real time PCR (Frisk *et al*., 1999) [1]. Kim *et al*. (2006) [3] study used RT-PCR to compare the various bodily secretions for the early detection of CDV in experimentally infected dogs. Maneesh *et al*. (2023) [4] has conducted the molecular detection and phylogenic analysis of CDV in dogs across Wayanad. The present study involves detection of CDV in dogs with respiratory signs by RT-PCR.

2. Materials and Methods
2.1 Collection of samples
A total of 15 deep nasal swabs were collected from dogs exhibiting high fever and respiratory signs suggestive of CD. Collected swabs were stored in RNA later at -20 ºC until use.

2.2 Extraction of RNA and conversion to cDNA
The RNA was extracted from nasal swabs using TRIZOL reagent and converted to cDNA by using commercial cDNA conversion kit (Bio-Rad). The cDNA was stored at -20 °C until use.

2.3 Reverse transcription-polymerase chain reaction
The primers targeting *N* gene of CDV were designed using Primer 3 software and custom synthesised from Sigma-Aldrich.

RT-PCR revealed amplicons of approximately 287 bp on gel documentation.

The forward primer *CDF*: ACAGGATTGCTGAGGACCTAT and reverse primer *CDR*: CAA GAT AAC CAT GTA CGG TGC were used. Multicomponent vaccine NOBIVAC-6 was used as positive control. Upon gradient PCR, annealing temperature was found to be at 58.1 °C

The PCR conditions for *N* gene includes: initial denaturation at 95 °C for 2 min, 35 cycles of denaturation at 95 °C for 30 sec, annealing temperature at 53 °C for 30 sec and extension at 72 °C for one min followed by final extension at 72 °C for 5 min.

2.4 Phylogenetic analysis

Sequence manipulation suite was used to convert the DNA sequence into its reverse complement counterpart. Two overlapping nucleic acid sequences were merged using EMBOSS merger.

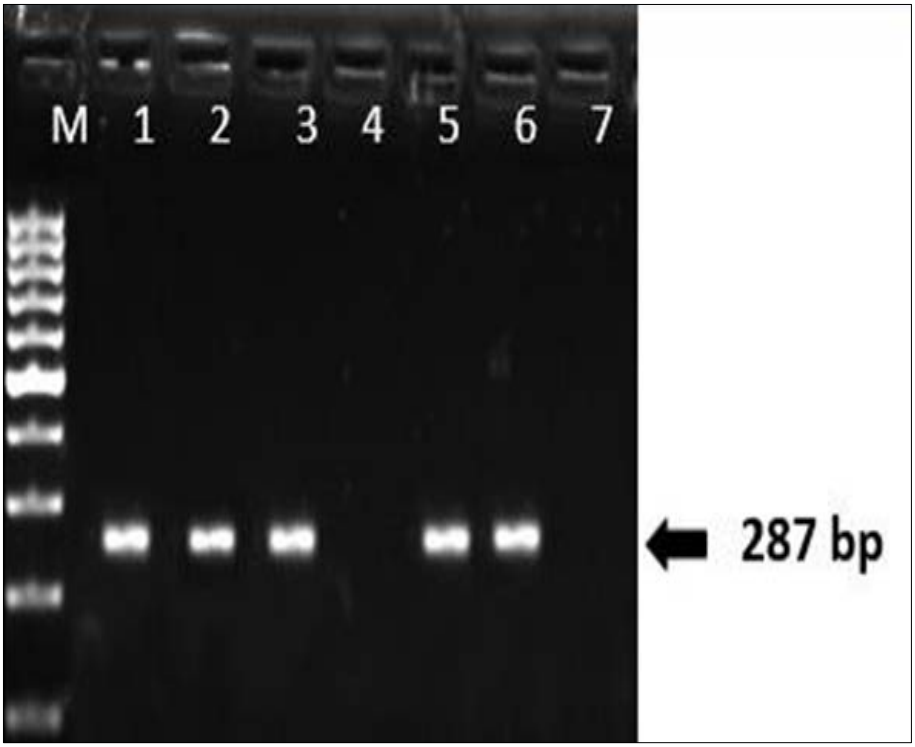
Transeq (EMBOSS) was used for translating nucleotide sequence to corresponding peptide sequence. Retrieval of related sequences using GenBank and confirmation of related sequences was done with NCBI BLAST. Phylogenetic tree was constructed using MEGA 11 software by employing neighbor joining tree method with bootstrap value 1000.

2.5 Results

Among the 15 samples tested, four were positive for CDV. Amplicons of approximately 287 bp was detected in the positive samples (Fig.1). Representative amplicon was submitted for sequencing.

Phylogenetic analysis

Confirmation of the sequences was done by BLAST analysis and was compared with related nucleotide sequences retrieved from GenBank. In the present study, phylogenetic analysis revealed that the sequence obtained were closely related to the sequence from Brazil and found to be in the sister clade of those from Hungary and China. However, they were not related to the isolate from Kerala or any other Indian isolates.



Lane M -100 bp ladder
Lane 1 - Positive control
Lane 7 - Negative control
Lane 4 - Negative sample
Lane 2, 3, 5, 6 - Positive samples

Fig 1: Agarose gel electrophoresis of PCR amplified products of CDV

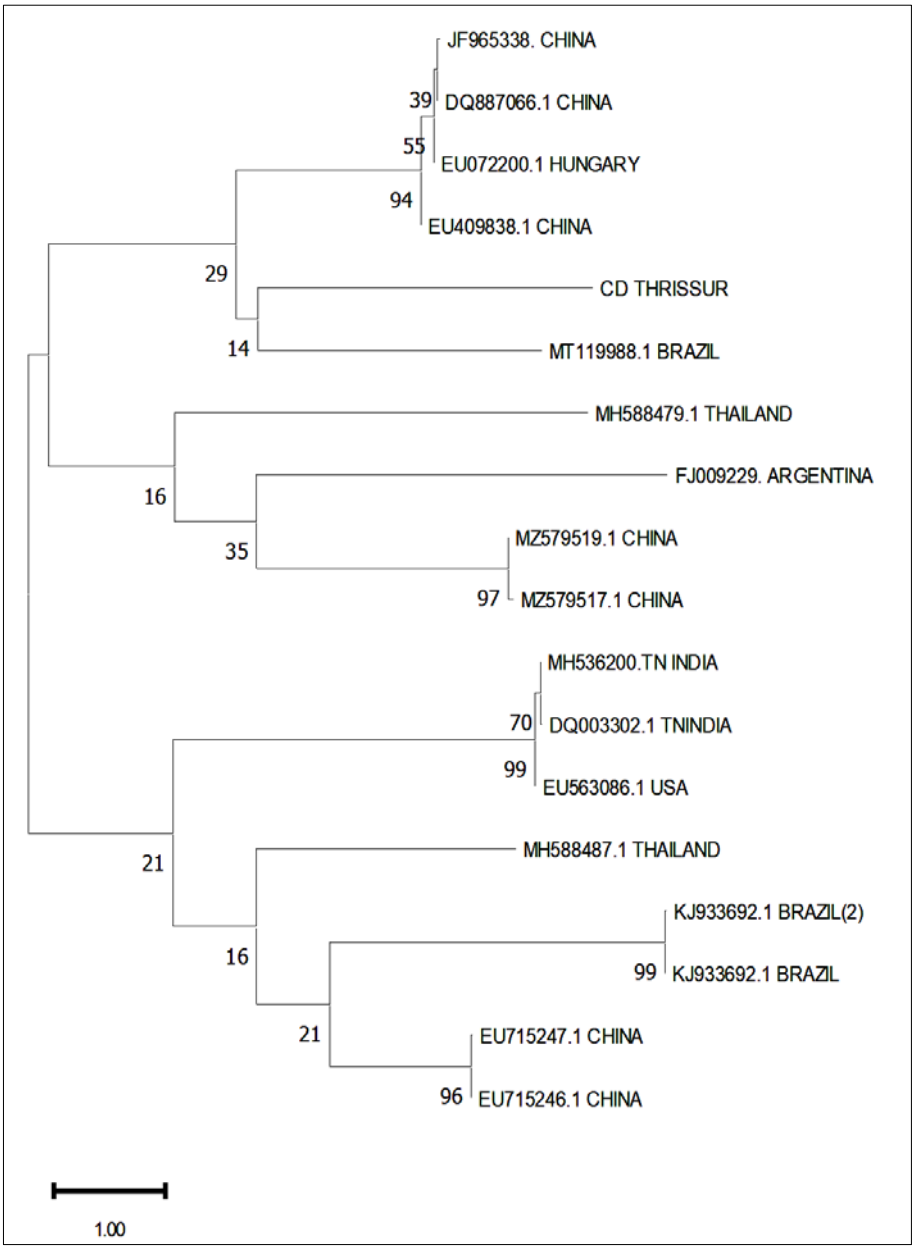


Fig 2: Phylogenetic tree based on *N* gene of Canine distemper virus

4. Conclusions

The *N* gene has been widely used to identify CDV because it is highly conserved. For the ante-mortem identification of CDV, RT-PCR was found to be a very sensitive and specific approach. Phylogenetic analysis indicated that the sequence obtained was closely linked to the sequence from Brazil and form sister clade of those from Hungary and China. They were, however, unrelated to the Kerala isolates or any other Indian isolates. A detailed study, involving more samples, spanning the entire state is warranted.

5. Acknowledgement

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