



NOTE

Virology

Phylogenetic analysis of *canine distemper viruses* isolated from vaccinated dogs in Wuhan

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ABSTRACT. *Canine distemper virus* (CDV) is an infectious agent that can cause canine distemper (CD), a lethal disease. Immunization is an effective method to control the infection; however, some cases of failed immunization are observed in animal hospitals every year. Therefore, in this study, we conducted phylogenetic analysis of the H gene of isolated CDVs. We first constructed a modified MDCK cell line, which constitutively expressed signaling lymphocyte activation molecule (SLAM), a specific receptor for CDV. The modified cell line was more suitable for propagation of CDV than the original MDCK cell line. Next, 9 CDVs were successfully isolated from 20 dogs with suspected CD-associated diseases. Of these CDV isolates, three were from vaccinated dogs. The analysis indicated that the H gene sequences of these 9 viruses were highly similar. The present study further supported the finding that the majority of CDV in China belonged to the genotype Asia-1, which was different from vaccine strains (America-1 and America-2). Although the clinical application of the vaccine suggested that it is effective against CDV infection, it remains an open question whether a novel vaccine based on the genotype of the Asia-1 strain would be more suitable for protection of dogs against Asia-1 CDVs infection.

KEY WORDS: canine distemper (CD), *canine distemper virus* (CDV), phylogenetic analysis, signaling lymphocyte activation molecule (SLAM), virus isolation

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Canine distemper (CD) is a highly contagious and fatal disease [2] that can cause severe acute systemic infections characterized by fever, coughing, vomiting, diarrhea, and neurological manifestations [6]. The species that can be infected include non-human primates, foxes, raccoon dogs, the giant panda, captive Siberian tigers and red pandas [3, 4, 12, 17, 18]. The pathogen responsible for the disease is *canine distemper virus* (CDV), which is a negative-sense, single-stranded RNA virus of the genus *Morbivirus* and the family *Paramyxoviridae* [13]. The H gene of CDV has the greatest genetic variation and is therefore widely used for investigating polymorphism in CDV isolates [3, 11, 13]. Based on the variability of the H gene, CDV strains can be grouped into at least 14 major geographically related genetic lineages: Asia-1, Asia-2, Asia-3, Asia-4, Europe, European wildlife, Arctic-like, Rockborn-like, America-1, America-2, Africa, South America-1, South America-2 and South America-3 [3, 11, 13].

CDV vaccines are very useful for controlling CD, but some cases of immunization failure are observed in animal hospitals every year. For example, our previous study demonstrated that 19% of CD cases came from vaccinated dogs in our animal hospital in the years 2016–2017 [8]. Of course, there are some reasons for immunization failure, including using the wrong procedure for the vaccine, and a prior infection before the vaccine takes effect. However, there are still some cases from vaccinated dogs that cannot be easily explained. Therefore, in this study, we sought clues by conducting a phylogenetic analysis of the H gene of these isolated viruses.

Because the low efficiency of CDV isolation on Madin-Darby Canine Kidney Epithelial (MDCK) cell line restricted the study on the virus, we firstly constructed a cell line to facilitate isolation of CDVs. The gene encoding signaling lymphocyte activation molecule (SLAM), a specific receptor for CDV attachment [15], was cloned from the cDNA of isolated monocytes from the blood of dogs by using SLAM-P1 (5'-AACGGATCCGCCACCATGGATTCCAGGGGCTTC-3') and SLAM-P2 (5'-AGAGGGCGGAATTCTTAGCTCTCTGGAACGCAC-3') primers. The amplified *slam* gene was cloned into the pRetro-TET3G vector (Takara Bio, Mountain View, CA, U.S.A.) at the *Bam*HI and *Eco*RI sites, yielding the plasmid pRetro-SLAM.

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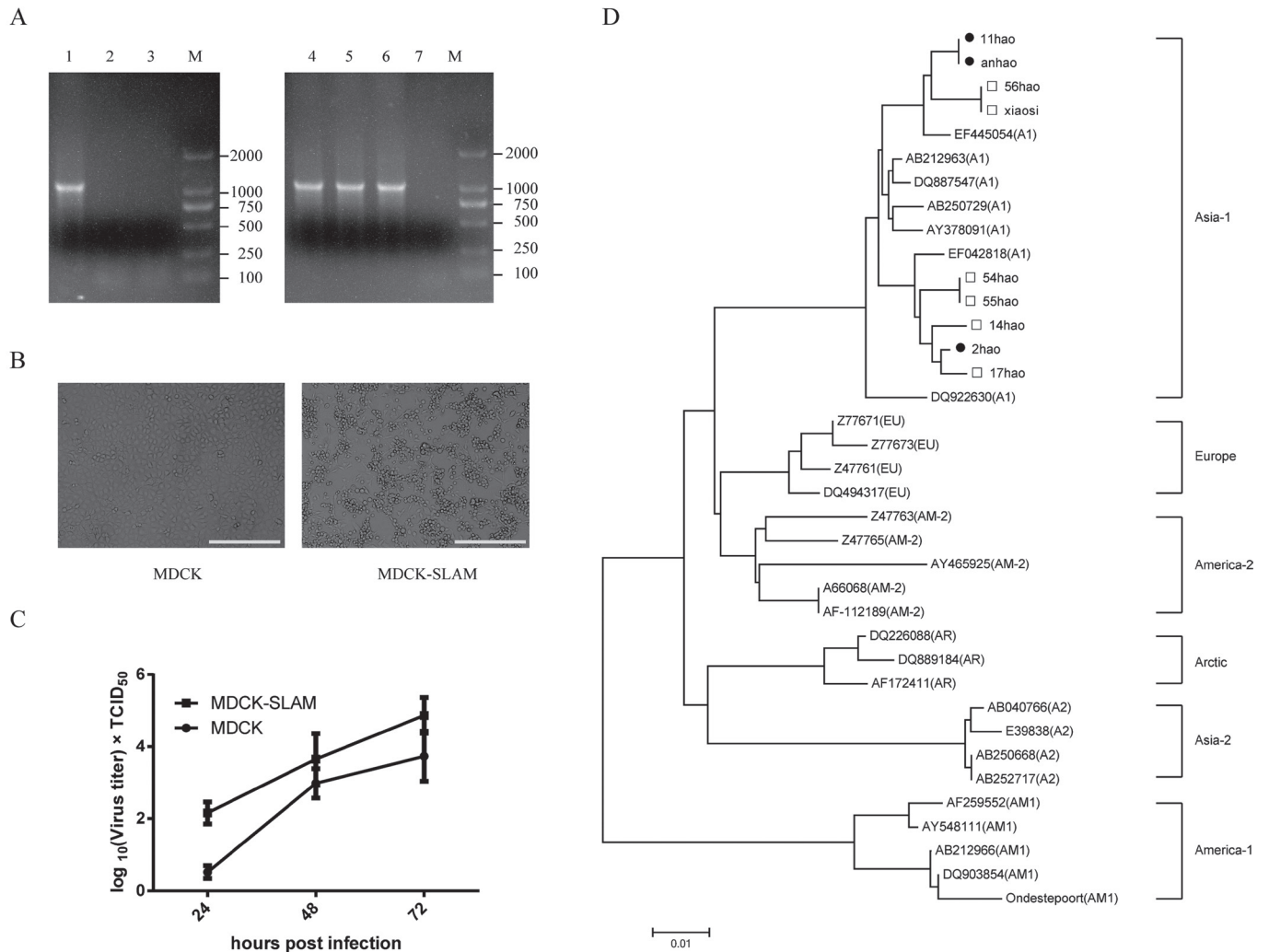


Fig. 1. Isolation of CDVs and analysis of their H gene sequences. **A.** Detection of the *slam* gene transcription in SLAM-MDCK or original MDCK. The total RNA was extracted from the SLAM-MDCK at the 1st (lane 1), 10th (lane 4), 15th (lane 5), and 20th (lane 6) passages or the original MDCK (lane 2), and then subjected for detection of *slam* transcription by RT-PCR with SLAM-P1/P2 primers. Lane 3 and 7 are a RT-PCR negative control. **B.** The image of MDCK and MDCK-SLAM inoculated with CDV strain AN-2 for 24 hr. Scale bar represents 50 μm . **C.** Comparison of the CDVs propagation curve on MDCK-SLAM cells and original MDCK cells at MOI=0.01. **D.** A phylogenetic tree of viral H gene sequences from the isolated CDVs using MEGAN software. The viruses isolated from vaccinated dogs or non-vaccinated dogs were marked by dark circle or rectangle respectively. Bootstrap values were calculated on 1,000 replicates.

pRetro-SLAM and pVSV-G were co-transfected into the GP2-293 cell line (Takara Bio) to yield Replication-Defective Retrovirus, which was further used to infect a MDCK cell line. Finally, the modified, SLAM-expressing MDCK cell line (MDCK-SLAM) was selected by using Geneticin™ Selective Antibiotic (G418 Sulfate) (Life technologies, Carlsbad, CA, U.S.A.). To confirm the expression of SLAM from the modified MDCK cell line, total RNA was extracted from MDCK-SLAM cells at the 10th, 15th, and 20th passages, and a RT-PCR was performed to detect the transcription of *slam* gene with SLAM-P1/ SLAM-P2 primers. Transcription of the *slam* gene fragments could be easily detected from the MDCK-SLAM cells at 10th, 15th and 20th passages, and it was not detected in the original MDCK cell line (Fig. 1A). These results suggest that transcription of the *slam* gene is stable in the modified MDCK cells.

To enable isolation of CDV in the MDCK-SLAM line, both the MDCK-SLAM line and the original MDCK cells were infected with CDV strain AN-2, which was isolated from a five-month-age dog in year 2010 and belonged to Asia-1. Infection of the MDCK-SLAM cells with CDV resulted in obvious Cytopathic effect (CPE) (i.e., unique syncytium), whereas no obvious CPE was observed on the original MDCK cells after 24 hr (Fig. 1B). Furthermore, the comparison of propagation curves of virus on MDCK-SLAM with original MDCK cells obviously indicated that MDCK-SLAM cells were better for the isolation of CDV than the original MDCK cells (Fig. 1C).

Using the MDCK-SLAM cells, we successfully isolated 9 CDVs from fecal and eye swabs of 20 dogs with suspected CD-associated diseases, which was also confirmed by RT-PCR assay. All efforts were made to minimize the suffering of the

dogs during collection of the samples. Interestingly, three CDVs (ANHAO, 11HAO and 2HAO) were from dogs that had been immunized with a commercial vaccine, and six CDVs (56hao, xiaosi, 54hao, 55hao, 14hao, and 17hao) were isolated from non-vaccinated dogs. The H genes of these viruses were cloned with primers: CDV-WH1 (5'-AACAAATGCTCTCCTACCAAGA-3') and CDV-WH2 (5'-AATGCTAGAGATGGGTTTATT-3') and then subjected for sequencing. The H gene sequences of three CDVs isolated from vaccinated dogs were submitted to NCBI database with accession numbers: MG922460, MG922458 and MG922459. These sequences were then used for phylogenetic analysis by using MEGA 6.0 software with a distance-based method (neighbor-joining) [14] (Fig. 1D). The results indicate that the sequences of the H genes from these viruses are highly similar, and they belong to the group Asia-1, which was different from the vaccine strain (America-1 and America-2). Therefore, the main genotype of CDVs prevalent in Wuhan in 2006–2007 was Asia-1.

In fact, CDVs have been categorized into many genotypes based on the H gene, and the genotypes are consistent with the geographic distribution of the viruses [3, 10, 11, 13]. The major vaccine strains of CDV were isolated from the 1930s through the 1950s (old CDV isolates from the United States; American-1 lineage) and have been used as CDV vaccines worldwide (Onderstepoort, Snyder Hill, and Lederle strains). In 1991, researchers noticed that the prevalent CDV strain in Japan belonged to genotype Asia-1 [16]. This genotype has been identified as a major genotype in many Asian countries, including Korea, Thailand, China, and Vietnam [1, 7, 9, 11]. Because the clinical data indicated that the majority of CD cases were from non-vaccinated dogs, it was thought that the strain (America-1 and America-2) used to make the vaccine was adequate for controlling the infection [5]. However, some clinical cases from vaccinated dogs still appear in animal hospitals. Thus, it remains to be further elucidate whether a novel vaccine based on the Asia-1 strain was better for protecting dogs against Asia-1 CDVs infection.

CONFLICT OF INTEREST. The authors declare that they have no competing interests.

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