Full Length

CHARACTERIZATION OF VACCINE ASSOCIATED STRAINS OF *GALLID ALPHAHERPESVIRUS1* CIRCULATING IN NAMAKKAL, TAMIL NADU

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ABSTRACT

Infectious laryngotracheitis (ILT) is an acute respiratory infection of poultry causing significant morbidity and mortality. Until recently, vaccines were not commercially available in India. Studies on molecular characterization of Gallid alphaherpesvirus1 (GaHV1) circulating in Tamil nadu revealed that the field strains are closely related to vaccine strains. In the present study, tracheal samples were collected from poultry suspected for ILT in the Namakkal region. Presence of GaHV1 infection was confirmed by PCR targeting ICP4 gene. Further, histopathological changes in the tracheal tissues were suggestive of ILT infection. Nucleotides of ICP4 gene were determined by Sanger sequencing, submitted to GenBank and accession numbers were obtained. Phylogenetic analysis of the ICP4 sequences revealed that the viruses circulating in the Namakkal region are related to Chicken Embryo Origin vaccine strains of GaHV1.

Key words: *Infectious laryngotracheitis, vaccine, poultry, gallid alphaherpesvirus1*

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INTRODUCTION

Infectious laryngotracheitis (ILT) is a highly contagious, acute respiratory disease of poultry caused by Gallidalphaherpesvirus 1 (GAHV1). In recent decades, emergence of ILT has been reported in commercial laying hens characterized by respiratory signs such as gasping (pump handle respiration), expectoration of bloody exudate, dyspnoea, and mortality ranging from 5 to 70% (Gowthaman *et al*., 2020; Ponnusamy *et al.,* 2022; Senthilnathan *et al*., 2024). In addition to mortality, economic losses due to loss of egg production, poor weight gain have a significant impact on the poultry industry (Garcia, 2017).

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Infection in poultry can be effectively prevented by vaccination. Two types of live attenuated vaccines are commercially available worldwide, Tissue Culture Origin (TCO) and Chicken Embryo Origin (CEO). However, it has been reported that often vaccinated flock suffer from diseases due to reversal of virulence and the emergence of new recombinant viral strains. Fowl pox virus and turkey herpes virus vectored vaccines carrying immunodominant proteins of GaHV1 are also commercially available (Elshafiee *et al.*, 2022). Several studies have reported that recombinant GaHV1 virus and CEO vaccine-like virus are causing outbreaks in different parts of the world (Bayoumi *et al.*, 2020, Ponnusamy *et al.*, 2022; Senthilnathan *et al.*, 2024). In addition, use of different types of vaccines in a flock can leads to superinfection and genomic recombination between the vaccine viruses and field viruses(Fakhri *et al.*, 2020). In the present study, we characterized the ICP4 gene of GaHV1 circulating in the Namakkal region, Tamil nadu to identify the source of outbreak in a area where suitable vaccination is practiced.

MATERIALS AND METHODS

Sample collection

A total of 11 tracheal samples were collected from layer submitted for post-mortem at Poultry disease diagnosis and surveillance laboratory, Namakkal during the month of March, 2025. The famers reported a 5-10% decrease in egg production and 10-20% mortality among different age group of layers. None of the farms reported that they vaccinated the flock with ILT vaccine. Portions of the tracheal samples were collected separately for histopathology in 10 % buffered formalin and for PCR stored in -20°C transport container.

Polymerase chain reaction

Initially tracheal tissues were ground using a pestle and mortar and subsequently subjected to three cycles of freeze thawing. Total DNA was extracted from the tissue homogenate using QIAamp DNA Mini Kit (QIAGEN, Germany) by following the manufacturer's protocol. The quality and quantity of DNA extracted was determined by NanoDrop spectrophotometer (Thermo Scientific, USA) and agarose gel electrophoresis.

Presence of *Gallid alphaherpes virus*1 infection was confirmed by PCR targeting a transcriptional regulator gene, ICP4. The primer sequences and expected amplicon size are presented in Table 1.

Histopathology

Tracheal tissues were subjected to histopathological examination to confirm the presence of inclusion bodies and other pathological features suggestive of ILT. The tissue sections were stained with H&E and observed under 100x oil immersion (Suvarna *et al.*, 2017).

Phylogenetic analysis

PCR amplicons of ICP4 were gel electrophoresed and purified using QIAquick Gel Extraction Kit (QIAGEN, Germany) by following manufacturer's instructions. The purified PCR amplicons were sequenced in both directions using Sanger dideoxy method and results were analysed using BioEdit software. The sequences were deposited in GenBank and accession numbers were obtained. Further, BLASTn analysis performed, sequences with more than 99 % query coverage were retrieved from NCBI database. Multiple sequence alignment was performed using ClustalW algorithm in Mega12 tool and a phylogenetic tree was constructed using maximum-likelihood method.

RESULTS AND DISCUSSION

PCR targeting ICP4 gene and Sanger sequencing

Detection of GaHV1 infection in poultry by PCR has been reported to be accurate, and rapid (Johnson *et al*., 1995; Puvarajan *et al*., 2017; Ponnusamy *et al.,* 2022). ICP4 and TK genes are commonly used to identify GaHV1 infection in field samples. Additionally, glycoprotein G, glycoprotein E and UL47 have also been used to detect GaHV1 infection and the nucleic acid sequences of these gene can be used to differentiate vaccine and field strains (WOAH, 2021).In the present study, genomic DNA was extracted from and detected the presence of GaHV1 infection was confirmed by PCR targeting ICP4 gene. ICP4 specific amplicon of 635 bp in size was found in 8 out of 11 samples (Fig. 1). One of the positive samples was subjected to Sanger sequencing and the nucleotide sequence was submitted to GenBank and an accession number was obtained (PV567551.1).

Histopathological examination of tracheal tissue

Histopathological examination of tracheal tissues revealed that lumen with large amounts of necrotic cellular debris and fibrin. The tracheal mucosa showed diffused erosion to ulceration of pseudostratified epithelium and large, angular multinucleate syncytia were observed within the mucosa. Ulceration extended into the lamina propria and submucosa with complete loss of goblet cells and projection of capillaries into the lumen. Syncytial cells contained up to 15-20 nuclei and eosinophilic nuclear inclusion bodies with chromatin margination were observed in many nuclei (Fig.2 and 3). Formation of syncytia along with intranuclear inclusion bodies is commonly reported in infectious laryngotracheitis affected trachea (Carnaccini *et al*., 2022; WOAH, 2021). The submucosa was moderately expanded by congested blood vessels and multifocal moderate to severe lymphocytic infiltrates. Sivaseelan *et al.* (2014) also reported that during the early stages of infection congestion of mucosal and submucosal blood vessels in the mid-tracheal region.

Phylogenetic analysis

Highly similar sequences were retrieved from NCBI database using BLASTn analysis of the partial ICP4 sequence obtained in this study. A total of 31 sequences were retrieved including TCO, CEO vaccine strains, field strains from different countries and sequences previously submitted from Tamil nadu, India. Phylogenetic analysis revealed that the partial ICP4 sequence from this study clustered with CEO vaccine strains of GaHV1 (Fig. 4). Ponnusamy *et al*. (2022) also reported a similar finding that ICP4 sequences of GaHV1 circulating in Tamil nadu state were closely related to the CEO vaccine strains and CEO reverent strains from different. Field isolates from Canada exhibited 99.97 % nucleotide similarity with the LT Blen® vaccine strain sequences (Elshafiee *et al*., 2022). These findings indicate that field isolates from a particular geographical region are often closely related to vaccine strains of virus used in that region.

Further, the ICP4 sequences of GaHV1 isolated over a period of 10 years in Tamil nadu state were clustered together. Low mutation rate and genetic stability of the GaHV1 is attributed to this clustering (Piccirillo *et al*., 2016). Nucleotide sequence of TK, glycoprotein C, D and UL47 have revealed that GaHV1 isolates in different regions of Tamil nadu are closely related to CEO vaccine strains (Ponnusamy *et al*., 2022; Senthilnathan *et al*., 2024). Collectively, these findings suggest that GaHV1 circulating in the region may share a common ancestor.

In conclusion, Gallid alphaherpesvirus1 isolates circulating in Tamil nadu, India were closely related to CEO vaccine strains. These findings suggest that outbreaks of infectious laryngotracheitis in poultry may be associated with CEO revertant or recombinant viruses that have emerged over a period of time.

Table 1: Details of the oligonucleotides used in this study

Gene	Oligonucleotide sequence	Amplicon size	Reference
ICP4	Forward 5’-CTTCAGACTCCAGCTCATCTG-3’	635 bp	Chacon *et al*., 2010
	Reverse 5’-AGTCATGCGTCTATGGCGTTGAC-3’		

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CONFLICT OF INTEREST

Authors have no conflict of interests.

Ethical approval: Not Applicable.

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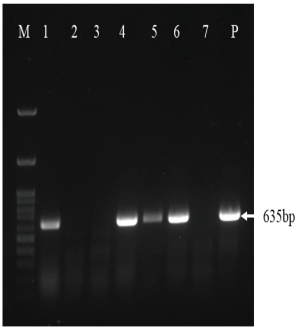

Fig.1. PCR amplification of GaHV1 ICP4 gene. Lane M: DNA ladder, Lane 1, 4-6: Positive field samples showing specific amplicon of 635 bp, Lane 2,3,7: Field samples negative for ICP4, Lane P: Positive control

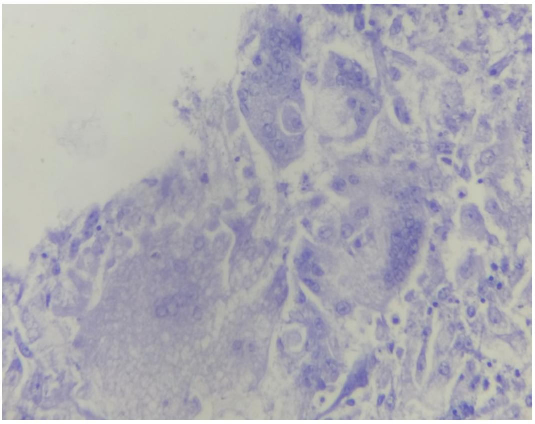

Fig.2. Tracheal mucosa with formation of multiple large, angular multinucleate syncytia within the mucosa and within sloughed luminal debris (H&E 40x)

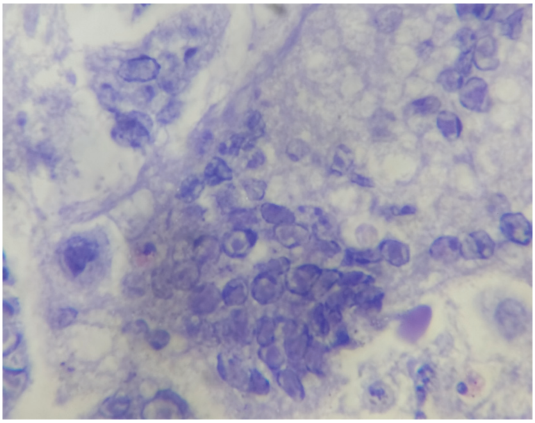

Fig.3. Tracheal tissue showing syncytia with many nuclei contain large, eosinophilic nuclear inclusion bodies that marginate chromatin (H&E 100x oil immersion)

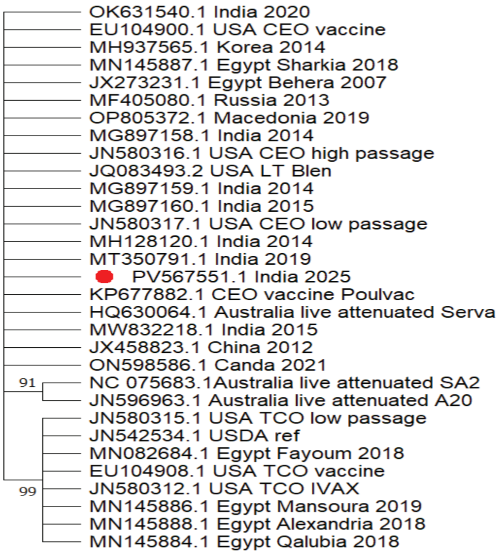

Fig. 4. Phylogenetic tree constructed by Maximum-likelihood method with 1000 bootstrap replicates using Tamura 3-parameter model (MEGA 12 tool). ICP4 sequence of the GaHV1 used in this study was analysed using other sequences of vaccine and field isolates available in NCBI database.

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