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Characterisation of avian paramyxovirus - 1 genotype IV infection in pigeons in northern Tamil Nadu

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Abstract

Avian paramyxovirus type 1 (APMV-1), the causative agent of Newcastle disease, is an important pathogen of domestic and wild birds. The present study investigated APMV-1 infection in pigeons from Irumbedu, Kanchipuram District, Tamil Nadu. Four moribund pigeons showing torticollis, tremors, upward-looking posture, and wing paralysis were examined during August 2024. Tissue samples were collected and processed for viral RNA detection, followed by RT-PCR targeting the complete fusion (F) gene. All four samples were positive for APMV-1, and the F gene sequence of one sample was subjected to Sanger sequencing and phylogenetic analysis. The sequence showed 96.88% nucleotide identity with a pigeon-derived APMV-1 isolate from Tamil Nadu reported in 2000. Phylogenetic analysis revealed that the Kanchipuram 2024 strain clustered within genotype IV. The findings demonstrate the presence of genetically diverse pigeon-associated APMV-1 strains in India and emphasize the need for continued surveillance and biosecurity to reduce the risk of transmission between pigeons and poultry.

Keywords: APMV-1, Pigeon paramyxovirus, Newcastle disease, Pigeons, Fusion gene, RT-PCR

1. Introduction

Avian paramyxovirus type 1 (APMV-1) is an important viral pathogen of domestic and wild birds and is the causative agent of Newcastle disease (ND). APMV-1 belongs to the genus *Orthoavulavirus* within the family *Paramyxoviridae* (Dimitrov *et al.*; 2019) [3]. The virus is an enveloped, single-stranded, negative-sense RNA virus with a broad host range that includes chickens, turkeys, pigeons, doves, and numerous wild bird species. Although Newcastle disease is primarily recognised as a major disease of poultry, infection with APMV-1 in pigeons represents an important veterinary and epidemiological concern because pigeons can become infected, shed virus, and potentially contribute to the maintenance and transmission of particular APMV-1 strains among other avian populations, including chicken (Annaheim *et al.*, 2022) [1].

Pigeons are frequently infected by pigeon-associated variants of APMV-1, commonly referred to as pigeon paramyxovirus type 1 (PPMV-1). PPMV-1 has been reported from many countries (Annaheim *et al.*, 2022; Nooruzzaman *et al.*, 2021; Tian *et al.*, 2020) [1, 8, 9]. Neurological signs are particularly characteristic of PPMV-1 infection and may include depression, incoordination, torticollis, tremors, circling, paralysis, and difficulty in maintaining balance. Respiratory and gastrointestinal signs may also occur, including nasal discharge, coughing, diarrhoea, reduced appetite, and weight loss. In severe infections, mortality may occur, although disease severity can vary considerably among outbreaks. The virus can spread through direct contact with infected birds as well as indirectly through contaminated feed, water, equipment, housing, clothing, and other fomites. Wild and other free-ranging pigeons may facilitate the movement of virus between domestic, captive, and wild bird populations. The ability of infected pigeons to shed virus, sometimes before obvious clinical disease develops, is particularly relevant to disease control.

APMV-1 infection in pigeons is important not only because of its effects on pigeon health but also because of its implications for poultry and wild bird populations (Annaheim *et al.*, 2022) [1]. Effective control depends on early detection, appropriate biosecurity, prevention of contact

between infected and susceptible birds, hygienic management, and vaccination where appropriate. Continued surveillance and molecular characterisation of pigeon-associated APMV-1 viruses are therefore essential for understanding their evolution, geographical distribution, transmission dynamics, and potential contribution to the epidemiology of Newcastle disease. In this study we report an incidence of APMV-1 infection in pigeons in northern Tamil Nadu.

Materials and Methods

Sample Collection

Four pigeons moribund condition were collected in Irumbedu, Kanchipuram District during August 2024. The pigeons died shortly after they were collected. Necropsy was performed and trachea, lungs, proventriculus, gizzard and small intestine were collected separately from each pigeon for virological studies and stored in -20 °C freezer until further processing.

Isolation of Viral RNA

The tissue samples collected from the pigeons were separately processed using a sterile mortars and pestles in an aseptic Biosafety Cabinet cleaned with RnaseZap (Thermo Fisher). RNA was isolated from the samples by trituration of the samples in sterile Minimum Essential Media with antibiotics and without serum. The RNA isolation was performed using Trizol reagent (Thermo Fisher) according to the manufacturer's protocol. The isolated RNA were stored in -80 °C freezer until further processing.

Reverse Transcription Polymerase Chain Reaction and sequencing

First strand complimentary DNA (cDNA) was synthesized by reverse transcription from the sample RNA using random hexamers and iScript™ cDNA synthesis kit (BioRad). The cDNA was subjected to polymerase chain reaction (PCR)

using Pfu DNA Polymerase (Thermo) to detect the presence of APMV-1 viral genomic RNA and amplify the complete Fusion (F) gene and flanking region of the F gene using a set of primers; Forward - 5'-GTAGAAGATTCTGGATC-3' and Reverse - 5'-CCAGAGCTTTTCACATGG-3'. The primers amplified a region of approximately 1730 bases. The thermocycler conditions of 48 °C, 30 min; 94 °C, 1 min; followed by 40 cycles of 94°C, 15 s; 55 °C, 15 s; 68 °C, 2.5 min, followed by 68 °C, 5 min; and then held at 4 °C were used. The amplified gene was sequenced to characterise type of the APMV-1 detected by Sanger Dideoxy Sequencing with M/s Genoseq Diagnostics Pvt. Ltd., Chennai.

Phylogenetic analysis

The complete F gene sequence was compared with reference sequences using the MEGA12 software. The sequences were aligned by CLUSTAL W algorithm. Evolutionary analysis were inferred by the maximum likelihood method and Tamura parameter – 3 model with 1000 bootstrap replicates. The phylogenetic tree was further annotated using iTOL (iTOL: Interactive Tree of Life (embl.de)).

Results

Clinical Signs

Four pigeons were found in a moribund condition in a small field where free range desi chicken were reared in Irumbedu, Kanchipuram District during August 2024. The birds were suffering from torticollis, looked upwards, tremors in the head and body, and paralysis affecting the wings was observed. The symptoms were indicative of APMV-1 infection. Similar sightings of two more similar incidents affecting pigeons was reported by the farmer in the weeks prior to the sample collection.

Reverse Transcription Polymerase Chain Reaction

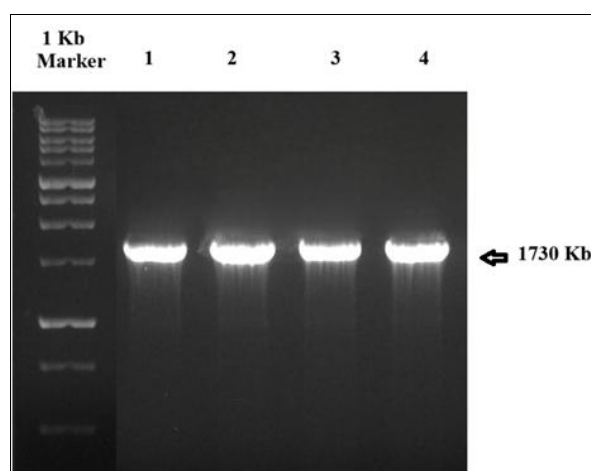


Fig 1: PCR assay to amplify the complete Fusion gene of APMV-1 including flanking regions (~ 1730 bp)

The RNA isolated from the four pigeon tissues were subjected to reverse transcription followed by F gene specific PCR as described in the material and methods. All the four samples were positive for the presence of the APMV-1 genome indicated by the amplification of the complete F gene in the PCR assay (Figure 1). The PCR amplicon from sample 1 was subjected to Sanger sequencing from both ends.

Phylogenetic analysis

The sequence of the amplicon of complete F gene was subjected to BLASTN search against the core_nt database to find highly similar sequences using the National Center for

Biotechnology Information website (<https://blast.ncbi.nlm.nih.gov/Blast>). The sequence of Newcastle disease virus isolate 2K3/Chennai/Tamil Nadu with accession no FJ986192.2 was found to be the best match with 96.88% nucleotide identity, which is a complete genome of APMV-1 virus isolated from pigeon in the year 2000. The sequence obtained in this study (Kanchipuram 2024 BTIS-MVC-TANUVAS) was then compared with five other complete F gene sequences of APMV-1 from pigeons in India along with ten reference sequences of complete F genes used for genotyping APMV-1 (Dimitrov *et al*; 2019) [3].

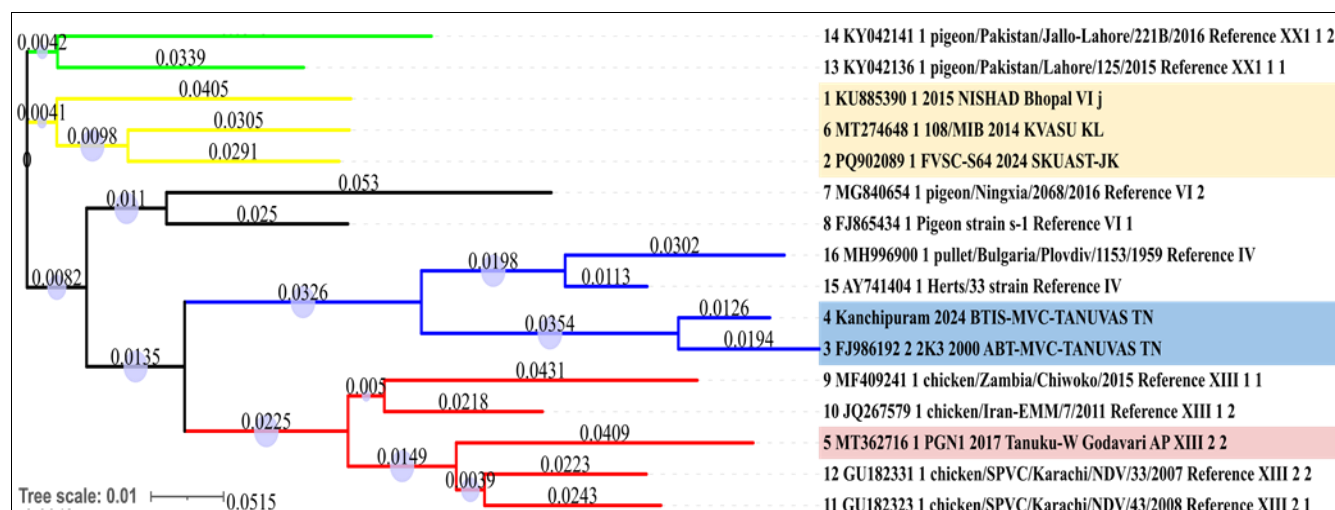


Fig 2: Phylogenetic analysis of the Kanchipuram pigeon APMV-1 sequence along with five other pigeon APMV-1 sequences from India and ten reference sequences used for genotyping APMV-1

The phylogenetic analysis of the sixteen sequences revealed that the Kanchipuram strain clustered with genotype IV along with another pigeon APMV-1 isolate from Tamil Nadu.

Discussion

Many PPMV-1 viruses belong predominantly to genotype VI of class II APMV-1, whereas many viruses historically associated with poultry Newcastle disease belong to other genotypes. There is also considerable cross-species infectivity. Pigeon-derived APMV-1 viruses can infect chickens, and experimental or natural infections can sometimes produce significant disease in poultry (Annaheim *et al.*, 2022; Gul *et al.*, 2026) ^[1, 6]. Conversely, poultry-associated APMV-1 viruses can infect pigeons. The clinical manifestations of APMV-1 infection in pigeons vary according to viral strain, infective dose, age and immune status of the bird, and other environmental or concurrent infections. One of the most important determinants of virulence is the fusion (F) protein cleavage site. Highly virulent Newcastle disease viruses generally possess a polybasic cleavage site that allows efficient activation of the F protein in many tissues. This contributes to systemic infection and severe disease. However, other viral genes, also influence the outcome of infection with APMV-1.

The F gene sequence of APMV-1 Kanchipuram strain collected in 2024 reported in this study clustered with genotype IV of APMV-1 typing scheme. In addition, the F gene of the Kanchipuram 2024 strain had the highest identity of 96.88% to another pigeon derived APMV-1 collected in 2000 reported from Tamil Nadu. The phylogenetic analysis also revealed that the fusion gene sequences of pigeon associated APMV-1 reported from the ICAR-National Institute of High Security Animal Diseases (NISHAD), Bhopal (2015) (Ganar *et al.*, 2017) ^[5], Kerala Veterinary and Animal Sciences University (2014) and SKAUST, Jammu and Kashmir (2024) (Gul *et al.*, 2026) ^[6] were closely associated with genotype XXI, whereas the NISHAD isolate (2015) was earlier classified under genotype Vij. The pigeon APMV-1 sequence from Tanuku, West Godavari district of Andhra Pradesh (Balam *et al.*, 2024) ^[4] clustered with genotype XIII.2. The analysis shows that pigeon strains in India belong to diverse genotypes from IV, VI, XIII and XXI. Pigeon paramyxoviruses are predominantly associated with genotype VI and XXI (Ather *et al.*, 2023; Hossain *et al.*, 2023; Nooruzzaman *et al.*, 2021; Tian *et al.*, 2020; Zhan *et*

al., 2020) ^[2, 7, 8, 9, 10]. However, the analysis of all the six complete F gene sequences of pigeon associated APMV-1 reported from India from 2000 to 2024 indicate a diverse distribution of the genotypes from IV, VI, XIII and XXI. The potential of the cross-species infections of APMV-1, especially between pigeons and chickens is a bio-security risk for chicken farming. This warrants increased surveillance for APMV-1 in feral birds such as pigeons and crows which frequently habituate close to chicken farms.

Conflict of Interest

Not available

Financial Support

Not available

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