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Paper



Genomic insight of avian reovirus circulating among desi-chickens in Tamil Nadu, South India

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Abstract

Avian reovirus (ARV) is a major causative agent of viral arthritis (VA), tenosynovitis, and malabsorption syndrome (MAS) in chickens, with significant economic consequences due to growth retardation, reduced production performance, and immunosuppression. Despite routine vaccination of breeder chickens against ARV, cases of VA and MAS continue to be reported in commercial flocks in recent years. Moreover, there is a lack of recent data on the genetic characteristics of circulating field ARV strains in India. In light of these concerns, a study was conducted to investigate the involvement of ARV in chickens exhibiting clinical signs suggestive of VA or MAS. Samples were collected from 27 commercial broiler and *desi*-chicken flocks across the mid-western region of Tamil Nadu, South India. Molecular confirmation was performed using reverse transcription polymerase chain reaction (RT-PCR) targeting a partial region of the σC gene within the S1 segment. Of the 27 flocks sampled, only two samples - both from *desi*-chickens aged two and three weeks - tested positive for ARV. Sequence analysis of these positive samples, compared against available ARV sequences in GenBank (including vaccine strains) revealed that the identified strains clustered within ARV genogroup II. This represents the first report of cluster II ARV in India, indicating the circulation of genetically distinct ARV strains in Indian poultry populations. These findings underscore the need for routine molecular surveillance of ARV genotypes in India and highlight the potential mismatch between circulating field strains and current vaccine strains. Comprehensive genotype monitoring is essential to upgrade vaccine design and implement effective control strategies for ARV-associated diseases in Indian poultry production.

Keywords

Avian reovirus, Cluster II, Desi-chicken, Viral arthritis, Malabsorption syndrome, RT-PCR

Introduction

Avian reovirus (ARV), a member of the genus Orthoreovirus within the family Spinareoviridae, is a non-enveloped virus measuring 70–80 nm in diameter with a characteristic icosahedral structure (King et al., 2012). The viral genome consists of ten segments of double-stranded RNA (dsRNA), each ranging from 1 to 4 kb in length. These segments are classified into three groups based on size: large (L1–L3), medium (M1–M3), and small (S1–S4). ARV encodes at least 12 proteins, including eight structural and four non-structural proteins (Benavente and Martínez-Costas, 2007). Among these, the S1 segment encodes the σC (Sigma C) protein, a minor capsid protein known for its high genetic variability. The σC protein mediates viral attachment to host cells and elicits virus-specific neutralizing antibodies, making it a key target for immune responses (Guardado et al., 2005).

ARV infection has been associated with a variety of diseases in both commercial and wild birds (Jones, 2000; Mor et al., 2014; Wang et al., 2019). The virus affects multiple organ systems, including the myocardium, digestive tract, respiratory tract, and central nervous system (Davis et al., 2012; Dandar et al., 2013). Notably, ARV is implicated in diseases such as runting-stunting syndrome (RSS) or malabsorption syndrome (Page et al., 1982), transmissible

proventriculitis (Kouwenhoven et al., 1978; Dormitorio et al., 2007; Pu et al., 2008), viral arthritis/tenosynovitis (Jones, 2000), and myocarditis (Jones, 2013). These conditions primarily affect young chickens and are transmitted either horizontally via the fecal–oral route or vertically from infected breeders (Mansour et al., 2018).

Currently, ARV vaccines used globally, including in India, are based on strains S1133, 1733, 2408, and 2177, all of which belong to genotypic cluster I (Chenier et al., 2014). Despite widespread vaccination of breeder flocks, there has been a resurgence in ARV-associated conditions such as tenosynovitis and MAS in commercial chickens, especially in young and growing birds, often resulting in poor growth and carcass condemnation (Kataria et al., 1983; Jones, 2013). Since 2012, an increase in ARV infections has been observed even among progeny of vaccinated breeders, suggesting that vaccine strains may not provide adequate cross-protection against emerging field strains due to genotypic divergence (Sellers, 2017).

Despite high seroprevalence rates (>90%) reported in Indian commercial layers during various periods (Kataria et al., 1983; Sawale et al., 2019), there is currently no molecular data available on circulating ARV genotypes in the state of Tamil Nadu or across India. Previous studies have demonstrated that the σC gene, located within the S1 segment and encoding the most variable structural protein of ARV, is a reliable marker for genotypic classification (Kant, 2003; Vasserman, 2004; Troxler et al., 2013). Given the role of σC in viral attachment and immune recognition (Guardado et al., 2005; Vasserman et al., 2004), sequencing and analysis of this gene is critical for understanding genetic diversity and upgrading vaccine strategies. Therefore, the present study aimed to genetically characterize ARV strains circulating among commercial poultry in the western region of Tamil Nadu, South India, by sequencing the σC gene of the S1 segment.

Materials and Methods

Collection of samples

A total of 27 chicken flocks exhibiting clinical signs of viral arthritis (VA), malabsorption syndrome (MAS), or runting-stunting syndrome (RSS) were screened for avian reovirus (ARV). These included 22 commercial broiler flocks and 5 *desi* (native) chicken flocks located in the districts of Namakkal, Karur, and Tiruppur in mid-western Tamil Nadu, South India (Figure 1). From each flock, 4 to 6 birds displaying clinical signs and found dead were selected for sampling. The average age of the flocks was 3.5 weeks, with a mean flock size of approximately 5,000 birds. The farms selected represented about 6% of the total farms in the region and were chosen randomly based on flock history and age. To minimize cross-contamination, all sampled farms were located at least one kilometre apart. All flocks were raised under a deep litter housing system for meat production.



Figure 1. A map showing various districts of Tamil Nadu, India. Courtesy: www.veethi.com. The underlined districts comprised the study area. Place of white triangles represents the farms' location.

The study was conducted during the post-monsoon to winter season (October to January) of the year 2024. Tissue samples (liver, heart, spleen, intestine, and pancreas) were aseptically collected from freshly dead birds, pooled per flock, placed into sterile containers, transported on ice to the laboratory, and stored at -80°C until further processing.

For virus isolation and RNA extraction, tissue pools were homogenized in sterile phosphate-buffered saline (PBS). The homogenates were centrifuged at $2,236 \times g$ (equivalent to 5,000 rpm in an 8 cm radius rotor) for 15 minutes. The resulting supernatant was filtered through a $0.45 \mu\text{m}$ syringe filter and used for subsequent RNA extraction.

Molecular identification of ARV by RT-PCR

Total RNA was extracted from the filtered organ supernatants using a commercial reagent (RNAiso Plus, Takara Bio, USA) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using the iScript™ cDNA Synthesis Kit (Bio-Rad, USA).

Polymerase chain reaction (PCR) was performed to detect ARV nucleic acid using thin-walled PCR tubes (200 μl capacity) in a final reaction volume of 20 μl . Each reaction contained: 10 μl of 2 $\times$ Taq Master Mix Red (Amplicon, USA), 1 μl of forward primer (10 pmol/ μl), 1 μl of reverse primer (10 pmol/ μl), 2 μl of cDNA (converted from RNA extracted from pooled organ samples), and 6 μl of nuclease-free water.

The primers used for amplification targeted a 940 bp fragment of the ARV σC gene, as previously described by Goldenberg et al. (2010). The sequences were:

Forward: 5'-TCMRTCRCAGCGAAGAGARGTCG-3'

Reverse: 5'-TCRRTGCCSTACGCAMGG-3'

Thermal cycling conditions were as follows: initial denaturation at 94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 2 seconds, annealing at 53°C for 20 seconds, and extension at 68°C for 45 seconds. A final extension was carried out at 68°C for 5 minutes, and the reaction was held at 12°C for 5 minutes (Labnet International Inc., USA). PCR products were subsequently analyzed by agarose gel electrophoresis and documented using a gel documentation system (Bio-Rad, USA).

Sequence analysis

The amplified S1 gene fragments were sequenced using the Sanger dideoxy method on an automated sequencer (Eurofins Genomics India Pvt. Ltd., Bengaluru).

Raw nucleotide sequences were edited and assembled using the EditSeq module of the Lasergene software package (DNASTAR Inc., Madison, WI, USA). Sequence homology was assessed through comparison with reference ARV strains retrieved from GenBank using the MegAlign program.

Phylogenetic relationships were inferred using the maximum likelihood method in MEGA version 11.0. The reliability of the resulting tree was evaluated via bootstrap analysis with 1000 replicates.

Results

In the present study, 27 flocks of commercial broilers and *desi*-chickens under four weeks of age, showing clinical signs suggestive of viral arthritis (VA), malabsorption syndrome (MAS), or runting-stunting syndrome (RSS), were investigated for the presence of avian reovirus (ARV) by molecular methods. Clinical signs, including reduced feed intake, poor weight gain, uneven growth, and characteristic "helicopter wing" posture (Figure 2), were observed in two *desi*-chicken flocks aged between 2 and 3 weeks. In one affected three-week-old *desi*-chicken, the liver showed marked discoloration and cholecystitis (Figure 3), while other organs exhibited no significant gross lesions except for mild splenic congestion in some birds from ARV-positive flocks.



Figure 2. Three-week-old desi chick infected with avian reovirus showing 'helicopter wing'.



Figure 3. Three-week-old desi-chicken positive for avian reovirus showing liver discolouration with cholecystitis.

The overall ARV prevalence in the study population was 7% (2/27 flocks). Although clinical signs indicative of VA or MAS were observed in several broiler and *desi*-chicken flocks, only two *desi*-chicken flocks tested positive for ARV by RT-PCR. Notably, the ARV prevalence among *desi*-chicken flocks alone was 40% (2/5 flocks). Both ARV-positive flocks were geographically proximal, located in the Tiruppur district of Tamil Nadu. Within these infected flocks, the proportion of clinically affected birds ranged from 1% to 2%.

Avian reovirus detection by RT-PCR

Based on previously published protocols, PCR targeting the σC region of the *S1* gene was employed to screen pooled organ samples for the presence of avian reovirus (ARV). Each reaction included a positive control and a negative template control to validate assay performance. Among the 27 samples collected from various chicken flocks in the study area, only two samples—both originating from *desi*-chicken flocks—tested positive for ARV (Figure 4).

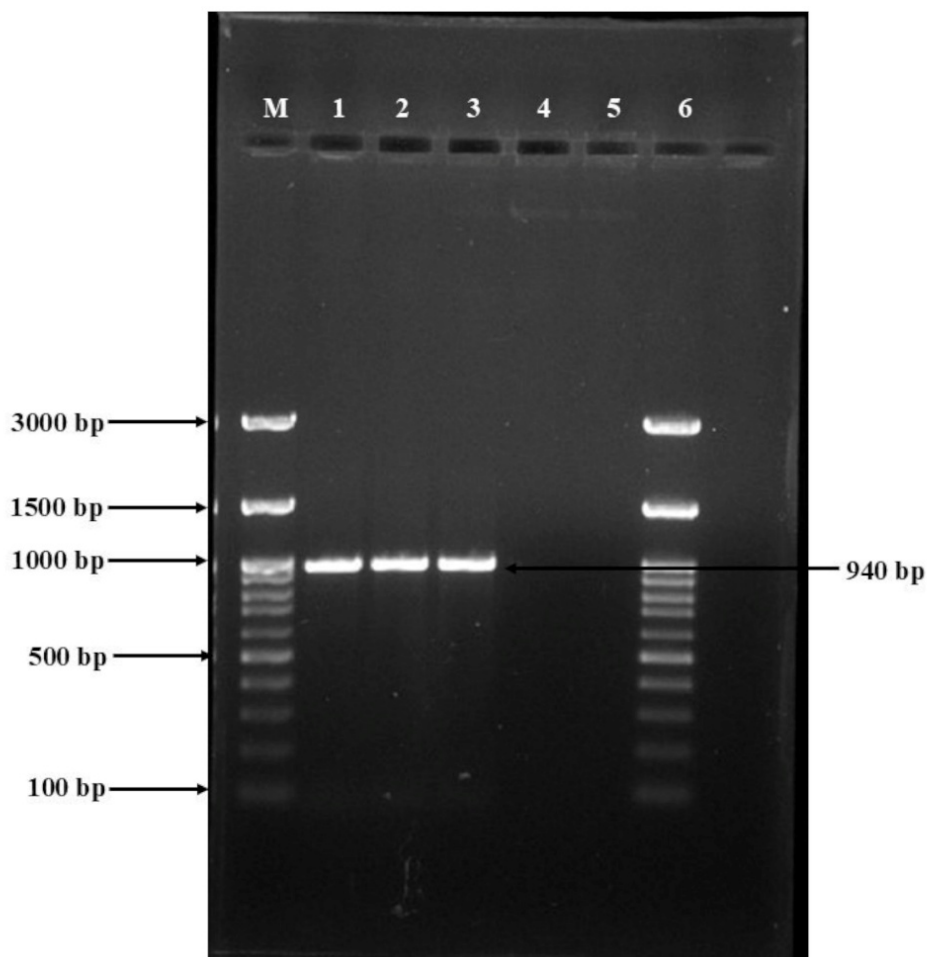


Figure 4. Agarose gel electrophoresis showing 940 bp amplified PCR product of σC part *S1* gene of field avian reovirus. Lane M and 6: 100 bp DNA marker; Lane 1: Two week-old desi-chicken flock; Lane 2: Three week-old desi-chicken flock; Lane 3: Positive control; Lane 4 : Negative control.

Molecular characterization of avian reovirus

Sequence and phylogenetic analysis

The nucleotide sequences of the two field ARV strains identified in this study (GenBank accession numbers: PQ240626 and PQ240627) were analysed via BLAST against the NCBI database. These two sequences shared a high nucleotide identity of 99.4% with each other. In contrast, the σC region of the *S1* gene from the field strains exhibited markedly low nucleotide identity (57.1–57.8%) with previously reported Indian ARV strains. Furthermore, the identity range with international strains varied widely, from 36.0% to 87.4%, reflecting considerable genetic diversity. Specifically, the highest similarity (87.4%) was observed with two Austrian strains (MZ520137 and MZ520138), while the lowest similarity (36.0%) was noted with a Hungarian strain (KX398238). Additional comparisons showed identity ranges of 36.9–85.4% with U.S. strains (n=16), 58.8–80.8% with Chinese strains (n=3), 56.5–66.9% with Israeli strains (n=2), 58.6% with a Canadian strain, 37.7% with a Dutch strain, 85.5% with an Italian strain, and 36.0–80.8% with other Hungarian sequences (n=3).

Phylogenetic analysis was performed using the maximum likelihood method in MEGA version 11, based on the σC region of the *S1* gene and incorporating 39 global ARV sequences, including nine from India. The tree topology, supported by 1000 bootstrap replicates, revealed the formation of six distinct clusters (Figure 5). The two field strains from this study clustered within group II, alongside 11 isolates from the USA, Austria, China, Hungary, Israel, and Italy. Notably, they displayed the highest sequence similarity with the Austrian strains MZ520137 and MZ520138. This constitutes the first report of cluster II ARV strains circulating in India.

Despite the low overall prevalence of ARV detected during the study period (7%; 2/27 flocks), the identification of two cluster II strains is significant. All previously reported Indian ARV sequences have clustered exclusively within group I,

underscoring the novelty and potential epidemiological importance of this finding.

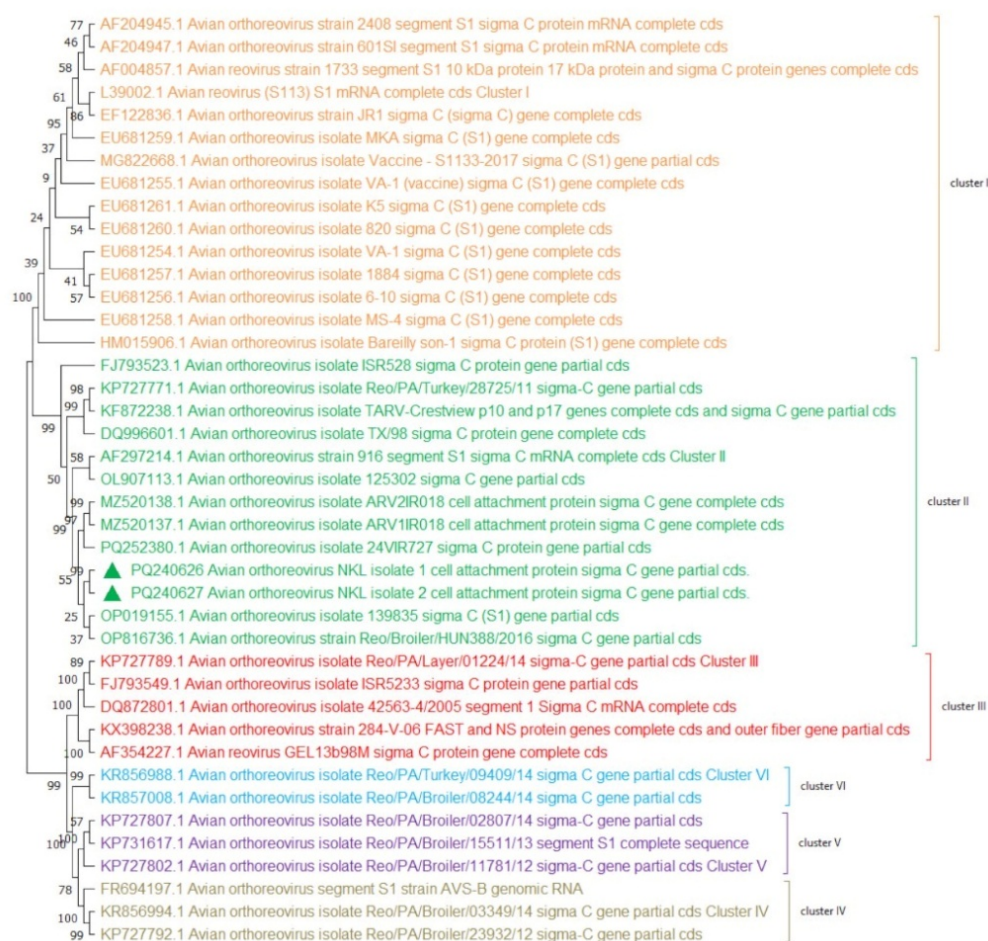


Figure 5. Phylogenetic analysis of σC part of *S1* gene of field strains with other published sequences using maximum likelihood method in Mega 11.0 with 1000 bootstrap values. Note: Two green arrows represent strains of the present study.

Discussion

The overall prevalence of avian reovirus (ARV) in the study area during the pre-winter and winter seasons of 2024 was low (7%). Although several flocks, including commercial broilers, exhibited clinical signs consistent with viral arthritis (VA) and malabsorption syndrome (MAS), they tested negative for ARV by RT-PCR. Alternative etiological agents such as *Staphylococcus aureus* and *Mycoplasma synoviae*, known contributors to arthritis and growth impairment in poultry, were not investigated in this study.

Notably, ARV positivity was detected in 40% of *desi*-chicken flocks (2/5), suggesting that the circulating strain may have acquired increased virulence, enabling infection in chickens typically considered more resistant to disease. It is important to note that parent flocks of these *desi*-chickens had not been vaccinated against ARV.

Conversely, broiler flocks presenting with signs of VA and MAS tested negative for ARV. These birds originate from breeder lines routinely vaccinated with inactivated vaccines derived from cluster I ARV strains. Despite the genetic mismatch between the circulating field strain (cluster II) and the vaccine strain (cluster I), ARV infection was not detected in commercial broilers during the study. This absence may be attributed to effective biosecurity measures and the lack of cluster II strain circulation in parent flocks.

The clinical signs observed in the ARV-positive flocks—including reduced feed intake, poor weight gain, uneven growth, and the characteristic "helicopter wings"—align with descriptions by Mirzazadeh et al. (2022). While other studies have commonly reported arthritis and tenosynovitis in affected commercial broilers (Palomino-Tapia et al., 2022; Sellers, 2022), the predominance of digestive signs in our cases may reflect strain-specific pathogenicity (Troxler et al., 2013; Lu et al., 2015).

RT-PCR was employed as a sensitive and specific diagnostic tool for ARV detection, targeting the σC gene segment of the *S1* genome. The σC protein is essential for viral attachment to host cells and is a key immunodominant antigen responsible for inducing neutralizing antibodies (Wickramasinghe et al., 1993; Kant et al., 2003; Vasserman et al., 2004). Due to its genetic variability and immunological relevance, the σC gene is frequently used for genotyping and molecular characterization of ARV (Schnitzer, 1985; Sellers et al., 2013; Lu et al., 2015; Gallardo et al., 2017).

Sequence analysis of the two ARV-positive field strains revealed a high degree of divergence from Indian reference strains (57–58% nucleotide identity), and broader variability (36–87.4%) when compared to global strains. These findings underscore the high mutation and recombination potential of ARV (Troxler et al., 2013; Lu et al., 2015).

Phylogenetic analysis grouped the two field strains within cluster II, alongside 11 international isolates from the USA, Austria, China, Hungary, Israel, and Italy. Notably, the closest relationship was observed with two Austrian strains (87.4% similarity). All previously reported Indian sequences were grouped in cluster I, making this the first report of cluster II ARV circulation in India.

Although no ARV-positive cases were detected in broiler flocks, it is noteworthy that maternally derived immunity in these birds would not protect against cluster II strains due to the absence of cross-protection among different ARV genotypes (Goldenberg et al., 2010). The localized and sporadic detection of cluster II ARV in *desi*-chickens may have limited its spread to commercial broiler populations during the study period.

Given the inherent genetic variability of ARV and the exclusive use of cluster I strains in current vaccines, these findings highlight the importance of ongoing molecular surveillance. Periodic genotypic monitoring of ARV strains in specific geographic regions is essential for designing effective vaccination and control strategies tailored to the circulating viral populations.

Conclusions

The present study documented the occurrence of ARV in 40% (2/5) of *desi*-chicken flocks reared intensively for meat production in the mid-western Tamil Nadu. RT-PCR proved to be an effective tool for the detection and molecular characterization of ARV. Sequencing and phylogenetic analysis of the σC gene segment of the *S1* gene revealed that both field strains belonged to cluster II. These strains exhibited low nucleotide similarity with previously reported Indian isolates but showed a close genetic relationship with two Austrian ARV strains. To the best of our knowledge, this represents the first report of cluster II ARV strains circulating in India.

Ethical approval

Not applicable.

Author contributions

Conceptualization: A.B., K.S.; Methodology: M.P., P.P., A.B.; Investigation: M.P., P.B.; Writing original draft: M.P., P.P.; Writing, review and editing: A.B., K.S.; Visualization: A.B., M.P., K.S.; Supervision: K.S., A.B., P.P., P.B. All authors have read and approved the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

All data are available on reasonable request to the corresponding author.

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