TRANSCRIPTOMIC PROFILING REVEALS MOLECULAR PATHWAYS IN NEWCASTLE DISEASE VIRUS INFECTED ASEEL CHICKEN EMBRYOS

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ABSTRACT

Transcriptomic study of lung tissue from Newcastle disease Virus (NDV) infected Aseel chicken embryos revealed 135 differentially expressed genes (DEGs) with p-values < 0.05. Subsequent study found that NDV infection caused significant changes in four Gene Ontology (GO) terms and 19 Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways. Understanding these GO enriched terms and KEGG pathways in the context of NDV-infected embryo is critical for better understanding the disease molecular processes and metabolic disruptions. This understanding underlines the virus's effect on host metabolism and immunological responses, which may help identify treatment targets or vaccine development strategies.

Keywords: Aseel, NDV infection, Gene Ontology, KEGG

INTRODUCTION

Newcastle Disease Virus (NDV) is a major threat to global food security, especially in developing nations where poultry is an important source of nutrition and income. In India, poultry production is a large sector, with a predicted 138.38 billion eggs produced in 2022-2023, with commercial poultry accounting for 118.16 billion (Source: BAHS Statistics 2023 DAHD, Government of India). NDV epidemics cause significant economic losses in poultry production (Charkhkar *et al.,* 2024). Poultry farmers are expose to additional hurdles due to socioeconomic issues such as restricted access to immunizations and veterinary services, especially among smallholder farmers in rural areas.

Indigenous Aseel chicken breed is recognized for its disease resistance and toughness under harsh environment, are still vulnerable to NDV (Yadav *et al.,* 2022; Radhika *et al.,* 2017). This breed contributes to genetic resources in backyard chicken husbandry, has better heat and disease tolerance potentials than commercial breed (Muthusamy *et al.,* 2024a; Malarmathi *et al.,* 2023**)**. However, contemporary poultry farming practices based on rapid growth have resulted in decreased immunocompetence in commercial breeds (Muthusamy *et al.,* 2024b).

Understanding the molecular pathways that drive NDV infection and immunological responses is critical for establishing effective preventative methods and treatment strategies. Analysing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in NDV-infected chickens, especially during embryonic stages, can provide useful information about immune system development and the regulatory genes involved. This study improves our understanding of how early viral infection affects immune responses in Aseel chicken.

MATERIALS AND METHODS

This study was conducted in strict accordance with the guidelines established by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. The experiments involving virulent Newcastle Disease Virus (NDV) were performed in Class 2-B2 cabinet facilities. All procedures were conducted with prior approval and supervised by the Institutional Biosafety Committee (IBSC) and the Institutional Animal Ethical Committee (IAEC) at VCRI, Namakkal (Approval Lr. No. 1764/VCRI-NKL/IBSC/2022, dated 11 May 2022, and Project Proposal No. 09/VCRI-NKL/2023, dated 8 September 2023) of TANUVAS, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India.

Sample Collection and RNA Sequencing

Six Aseel chicken embryos (19 days old), were obtained from the Department of Poultry Science at Veterinary College and Research Institute (VCRI) in Namakkal for a viral challenge study. In addition to a control group of three Aseel, the remaining three Aseel were inoculated with a lentogenic-B1 strain of live Newcastle Disease Virus (NDV) at a dosage of 106 EID50. Following inoculation, the embryos were incubated in an egg incubator at a temperature of 38 °C, with relative humidity maintained between 65 and 75 percent, until tissue harvesting at 24 h post-infection.

Lung tissue was collected from NDV-infected and control chicken embryos at 24 h post-infection. Tissues were immediately stored in *RNAlater*® (ThermoFisher) at −20 °C until RNA isolation. Total RNA was extracted using the Trizole method, using the RNAiso Plus, M/s Takara. The quantity of extracted RNA was measured using a nano-drop spectrophotometer, and RNA samples were combined at an equimolar ratio to form one pool for treated samples (three samples per pool), as well as one pool from control samples group. In order to verify the quality of RNA, a 2% electrophoresis gel was used to view the integrity of the extracted RNA. Before sequencing, quality and integrity of RNA was measured using the QubitTM RNA HS Assay Kit, in accordance with the manufacturer's instructions, on the Qubit 3.0 Fluorometer (Thermofisher Scientific, Vilnius, Lithuania)). Using RNA screen tape, the 4150 TapeStation system (Agilent) was used to evaluate the integrity of RNA. RNA with a RIN greater than 7 was recommended and proceeded for processing of RNA library. The estimated library fragment size comprised a wide peak with an average size of 350 bp and a range of 200–700 bp. The sample passed the quality check with more than 3 ng/μL, and more than 6 nM for the molarity concentration. Sequencing and data analysis were performed for deferential gene expression (DGE) and functional significance with the threshold of p-values < 0.05, for GO, KEGG, and gene cluster comparisons were performed using bioinformatic tools with the R package (version 4.2.1), such as Cluster Profiler, GOplot, and Pathview are complementary tools for functional analysis *(*Muthusamy *et al.*, 2024b).

RESULTS AND DISCUSSION

Transcriptomic analysis of lung tissue from NDV-infected Aseel chicken embryos identified significant ($p<0.05$) 135 differentially expressed genes (DEGs). Further investigation revealed that NDV infection caused significant ($p<0.05$) alterations in four Gene Ontology (GO) terms and 19 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Among these include one biological process that was associated with Gas Transport (GO: 0015669), while the remaining three cellular components included the Extracellular Region (GO:0005576), Extracellular Space (GO:0005615), and Ankyrin-1 Complex (GO:0170014). GO demonstrated that NDV caused functional alterations in immunocompetence of the studied chicken, particularly via altering the expression of two specific genes, Haemoglobin subunit epsilon 1 (HBBA) and Rh associated glycoprotein (RHAG), which were down regulated by -2.62 and -2.70 logFC, respectively (Figure 1).

Out of the 19 KEGG pathways (Figure 1), four exhibited corrected p-values less than 0.05, specifically Tyrosine Metabolism (gga00350), Metabolic Pathways (gga01100), Phenylalanine Metabolism (gga00360), and Nitrogen Metabolism (gga00910) indicating their statistical significance after adjusting for multiple testing. Further analysis of the KEGG pathway revealed that the genes 4-Hydroxyphenylpyruvate dioxygenase (HPD), Fumarylacetoacetate hydrolase (FAH), Glutamic-oxaloacetic transaminase 2 (GOT2), Carbonic anhydrase13 (CA13), and Carbonic anhydrase 2 (CA2) had a stronger association with NDV infection, with fold changes of 4.30 logFC, 3.03 logFC, 2.17 logFC, -3.35 logFC, and -2.64 logFC, respectively. These findings underscore the molecular mechanisms and metabolic disruptions induced by NDV infection, providing insights into the regulatory pathways involved in immune responses during early developmental stages.

Gene Ontology (GO) terms and KEGG pathways associated with Newcastle Disease Virus (NDV) infection in chickens showed significant metabolic shifts in biological processes and cellular components of the chicken embryo. Specifically, NDV infection causes changes in respiratory function, gas exchange, and cellular response in infected chicken embryos. The disruption in this metabolic pathway, influencing the host's physiological responses. This leads to altered energy production and nutrition alter protein synthesis and neurotransmitter levels, potentially impacting chickens' overall health and immunological responses (Muthusamy *et al.,* 2024b; Cheng *et al.*, 2022).

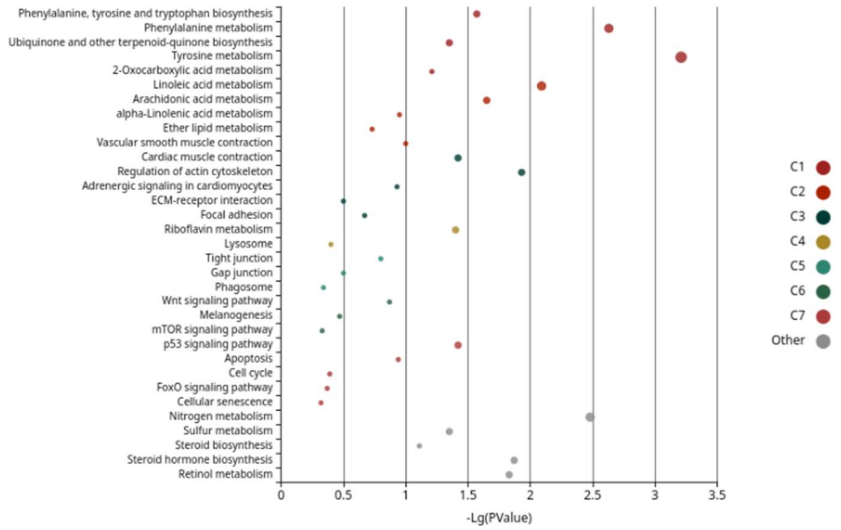

Figure 1. Illustration of key KEGG pathways in the lungs of NDV-infected chicken embryos.
(C legend colour indicated the cluster, and size indicated the number of genes)

Downregulation of the *HBBA* and *RHAG* genes in chickens can impair oxygen transport and disrupt acid-base balance, potentially leading to respiratory issues. This may negatively impact nutrient utilization, energy production, and overall health and development of the embryo (Fulton *et al.,* 2024; Kustu and Inwood, 2006).

CONCLUSION

Understanding the Gene Ontology (GO) terms and KEGG pathways related to NDV-infected chickens offers valuable insights into the molecular mechanisms of the disease metabolism and prognosis. This knowledge can help identify potential target genes, highlighting the virus's effects on host metabolism and immune response, which is crucial to therapeutic interventions and vaccine development. Additionally, further exploration of individual gene functions in the context of disease is essential for devising effective breeding strategies aimed at enhancing disease resistance and management within poultry populations.

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