A High Resolution Melting (HRM) analysis to differentiate VG/GA Avinew strain from virulent Newcastle Disease virus strains and pathotypes

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Introduction

Despite intensive vaccination programs, Newcastle disease virus (NDV) remains a constant threat to commercial poultry farms in Malaysia. Traditionally, NDV pathotypes (velogenic/mesogenic/lentogenic) are most commonly distinguished by sequencing and amino acid sequence analysis. However, with the rapid development of real-time PCR methods in recent years, there has been significant expansion to molecular diagnostics of various infectious agents through various novel methodologies [1]. Most importantly, a major concern in disease diagnostics is whether the disease outbreak is caused by a virulent field virus or due to a failed vaccination program. The present study describes a High Resolution Melting (HRM) curve analysis which distinguishes: 1) NDV pathotypes (lentogenic/mesogenic/velogenic) and 2) differentiates live vaccine strains (e.g. VG/GA Avinew strain) from virulent ND field virus (Differentiation of Infected versus Vaccinated (DIVA) assay). A primer spanning the F0 cleavage site of the fusion (F) protein gene was used to investigate its usefulness for characterizing the pathotypes present in NDV and investigating the DIVA assay.
Materials & Methods

Samples
A total of 14 samples that were confirmed positive for NDV by PCR and genetically characterized were used for this study.

DIVA (Differentiation of Infected versus Vaccine) assay & NDV Pathotype differentiation by High Resolution Melting (HRM) Curve analysis
The DIVA & HRM assay were established by using primer sets that amplify the hyper variable region of the fusion protein gene of NDV.
For both assay, the samples can be loaded into a 384-well microwell plate and subjected to PCR amplification in a real time PCR machine (LightCycler 480, Roche, Basel, Switzerland) followed by high resolution melting curve analysis.
1) The differentiation of the true NDV virus isolates versus the vaccine strains was achieved by using vaccines strains as positive controls and comparing their melting curve signatures.
2) The differentiation of the NDV pathotypes was achieved by using known positive NDV pathotypes as positive controls and comparing their melting curve signatures. Confirmation of the assay was done by sequencing the positive NDV isolates.
3) The assay was analyzed by using the Gene Scanning software supplied with the instrument. The clustering algorithm of the software allows all melting curves to be normalized and it subsequently clusters all samples and data that have the same melting signatures.
4) Sequencing of the fusion protein gene of NDV was done in a commercial sequencing facility using the BigDye® Terminator v3.1 cycle sequencing kit. A Basic Local Alignment Search Tool (BLAST) search of the sequence was done in the Genbank® database (Data not shown) to confirm the results. The sequence editing and assembly were done by using BioEdit® Sequence Alignment Editor version 7.0.5.2 208 (Tom Hall, USA). Sequences were aligned by using ClustalX™. The phylogenetic tree was constructed by using the distance-based neighbor joining method by using MegaTM 5 210 software (Biodesign Institute, Tempe, Arizona, USA) and evaluated using the bootstrapping method calculated on 1000 repeats of the alignment. The sequence identity matrix was generated with BioEdit® Sequence Alignment Editor version 7.0.5.2 (Tom Hall, USA).

Note: Data for phylogenetic tree & detailed amino acid sequence analysis are not shown.
Results

Differentiation of Infected versus Vaccinated Assay (DIVA)

Samples were compared with VG/GA Avinew strain. The wild-types can be visualized as the blue melting curves and the melting curves for the vaccine types are in red. The assay was analyzed by using the Gene Scanning software supplied with the instrument. The clustering algorithm of the software allows all melting curves to be normalized and it subsequently clusters all samples and data that have the same melting signatures.
Results

NDV pathotype differentiation by High Resolution Melting Curve

Samples were compared with a known vvNDV (velogenic). The NDV (velogenic/mesogenic) cases can be visualized as the blue melting curves. The green and red melting curves are positive lentogenic NDV cases. The Gene Scanning software from Roche was used for analyzing the data. The clustering algorithm of the software allows all melting curves to be normalized and it subsequently clusters all samples and data that have the same melting signatures. The accuracy of the results was determined by sequencing the controls.
Discussion & Conclusion

In this study, two diagnostic methods were developed:

1) Real Time PCR for the detection and differentiation of NDV pathotypes and
2) DIVA assay for Differentiation of Infected versus Vaccinated.

It was observed that the fusion protein gene exhibited the best results for use for the detection of NDV, differentiation of NDV pathotypes and DIVA assay compared to other NDV genes (L gene, M gene, HN gene, NP gene, P gene).

Validations were carried out to determine the sensitivity and specificity of the assay.

The results obtained correlated with the nucleotide sequencing analysis.

Our findings and results confirm that the two described methods that were developed fit for their intended use.

References: