Diagnostic Methods

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Comparison of classical and molecular diagnostic methods to resolve mixtures of Newcastle disease Virus pathotypes in one isolates

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Newcastle disease (ND) is a highly contagious disease of poultry, caused by an infection with Avian Paramyxovirus 1 (APMV-1, syn. NDV) and goes along with high morbidity and mortality in poultry. In commercial poultry holdings the disease can be successfully controlled by vaccination with live NDV strains of low virulence. Hence, efficient diagnostic of ND relies on a fast differentiation between vaccine and wild type virus. This can be achieved by determination of the proteolytic cleavage site of the fusion (F) protein. By definition, notifiable virulent NDV is characterized by multiple basic amino acids at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. Investigating NDV isolates from Egypt, a discrepancy between the molecular approach and classical biological pathotyping of isolates became apparent. Despite a proteolytic cleavage site of the F-protein corresponding to vaccine type virus, some isolates induced high mortality in chicks after intracerebral inoculation, classifying the isolates as virulent pathotypes. After cloning or enrichment of virulent NDV by cultivation in cell-cultures and subsequent sequencing a mixture of LaSota vaccine type and virulent NDV genotype VI was identified. This was verified by “next generation sequencing”. The results highlight the significance of mixed infections with regard to misinterpretation of sanger-sequencing results and demonstrate that deep sequencing is a powerful tool to unravel composition of virus mixtures.

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