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Easy, low cost, and precise Identification and interpretation of Newcastle virus and differentiation of its velogenic from lentogenic strains by using the nested RT-ARMS PCR

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Abstract

Newcastle disease is a viral disease of poultry that is caused by type 1 paramyxovirus, from the Avulavirus genus and its fast and precise diagnosis is of high importance. Virulence of this virus depends on Fusion protein (F), one of six proteins of this virus, which can be used for detection of the virus virulence. So, this study aims to design new primers according to bioinformatics science progression and suggest a cheaper and easier method for the identification of Newcastle virus (NDV) as well as distinguish lentogenic from velogenic strains with higher sensitivity and specificity. First, all available strains of Newcastle viruses were collected from NCBI data bank using Blast tool and after multiple alignments, universal and specific primers were designed. In the next step, identification of NDV was set up using universal primers by PCR on cDNA of the control positive sample. Then differentiation of lentogenic from velogenic strains set up by ARMS (Amplification Refractory mutation system)-PCR (Polymerase chain reaction) using specific primers. Because the method was performed on cDNA obtained from reverse transcription reaction (RT), and because the PCR product of the first PCR reaction was used as a template for nested second PCR reaction it is called "nested RT-ARMS PCR". Afterward, some samples from broiler farms were tested by this method and then compared by Real-Time PCR as a golden standard test. The results showed that sensitivity and specificity of identification of the virus and its strains were fully compatible in both methods. To sum up, this method which consumes a little bit more time but lower expenses, equipment and complexity in comparison with Real-Time PCR, can be suggested as a suitable substitution for the detection of NDV and distinguishing its velogenic from lentogenic strains.

Keywords: Newcastle, F protein, Velogenic, Lentogenic, Nested RT-ARMS PCR

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