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A new RT-PCR assay for the revealing of Newcastle disease viruses by designing a pair of universal primers

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Abstract

Newcastle disease viruses (NDVs) possess a single-stranded, non-segmented RNA and are classified into different strains based on their level of pathogenicity. Due to slight differences in the molecular makeup of the viral genome among these strains, employing a pair of primers for molecular diagnostics becomes essential. This study aims to establish a new approach to detect potential NDV infection by developing molecular methods. This was accomplished by performing a single RT-PCR reaction utilizing a newly designed universal primer set targeting a remarkably conserved area within the viral M gene. Various tools and resources were utilized to generate a set of primers, including the NCBI database and the Geneious Inspirational Software for Biologists. Ninety-four oropharyngeal swabs were collected from 66 chickens and 28 pigeons showing signs of ND. Viral RNA was extracted from samples, and M genes were amplified using conventional RT-PCR and real-time quantitative RT-PCR (qRT-PCR), followed by genomic sequencing and bioinformatics. The designed primers exhibited good quality, as indicated by a Delta G value of less than -5. This suggests that the primers are unlikely to cause any issues during the PCR process. Moreover, the amplification of the M gene was achieved successfully in both conventional RT-PCR and RT-PCR for approximately all collected samples from chickens and pigeons. This successful amplification was further verified through genomic sequencing and subsequent sequence analysis. These findings provide confirmation that the designed universal primers can effectively identify and quantify NDVs using PCR assay.

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Introduction

Newcastle disease (ND) is a highly infectious disease of poultry presenting a global challenge due to its effect on acute respiratory conditions and its association with neurological symptoms, depression, and diarrhea. This disease is a major worldwide threat to the poultry industry (1,2). Disease severity is determined by the susceptibility of the host and the virulence of virus strains. These factors contribute significantly to the severity of the disease (3,4). ND is commonly transmitted through direct contact between infected birds or birds that act as carriers. The infected birds release the viruses through their feces, contaminating the environment (5). The transmission of the disease can happen through direct contact with respiratory secretions and feces, contaminated water and food, and human clothing and equipment. NDVs can survive in the environment for several weeks, mainly in cold conditions (6). The disease is attributed to an avian paramyxovirus type 1(APMV-1) belonging to the Paramyxoviridae family. NDV is an enveloped virus with an unsegmented RNA genome and a helical capsid (7,8). The genetic material of NDV consists of six genes responsible for encoding six different proteins. These proteins include the nucleoprotein, matrix protein, fusion protein, phosphoprotein, large polymerase protein, and hemagglutinin-neuraminidase (fusion) protein (9,10).