Analysis of a Fragment of the Fusion Gene for Newcastle Disease by DNA Sequencing

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Abstract

Newcastle disease is caused by Avian Paramyxovirus Type 1 (APMV-1), which is found in the genus Avulavirus and family Paramyxoviridae (Suarez et al., 2020). Mild to severe respiratory, visceral, and central nervous system lesions contribute to the illness's devastating impact on vulnerable flocks.

The purpose of this study is to analyze a fragment of the fusion gene for Newcastle disease virus by DNA sequencing.

Alignment of the sequences of the three isolated (AN02, AN03 and AN07) against the registered sequences of Newcastle viruses in the database of NCBI were studied, and the result showed that, 94% identity with avian orthoavulavirus 1 isolate By Ch/IR/MAME96/2017, 99% identity with avian orthoavulavirus 1 isolate INDTNC22020 and 86% identity with avian orthoavulavirus 1 isolate INDTNC22020 respectively.

Virulent strain-specific motifs were found in the majority of circulating strains, as shown by partial sequencing of the F gene cleavage site.

Keywords: Fusion Gene, Newcastle Disease, DNA Sequencing.

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INTRODUCTION

Many different types of birds, including chickens, turkeys, and others, are susceptible to Newcastle Disease, a highly contagious and sometimes fatal illness. Depending on how dangerous it is to chickens, it is classified as either extremely pathogenic (velogenic), moderate (mesogenic), or mild (lentogenic) (Tips, 2017). There are two categories of ND virus velogenic strains that account for the virus's global distribution (class I and class II). Class I contains almost all of the low-virulence strains collected from wild ducks across the globe. Class II strains include both low- and high-pathogenic strains isolated from domesticated and wild birds (Birhan et al., 2019). The genus Avulavirus is where you'll find NDV; its part of the family Paramyxoviridae and the subfamily Paramyxovirinae. It is a negative-sense RNA virus with a single strand, like its close relatives (Bergfeld, 2017). In spite of the fact that, all NDV strains have a common serotype, they may be divided up into different groups based on their genetic makeup. On the basis of the whole sequence of the F gene, they have traditionally been categorized and described in terms of lineages and sublineages, classes, and genotypes (Tian et al., 2020). To clear things up, attempts have been made to identify the best global system of categorization. F gene phylogeny and evolutionary distances form the basis of the approach. Because it is more likely than other internal nucleocapsid genes to exhibit genetic variation, the fusion gene is often employed for comparative study (Susta et al., 2015). Rapid and accurate detection of Newcastle disease has been achieved with the use of molecular methods like RT-PCR and PCR (Naf'an et al., 2020). Negative and single-stranded RNA viruses may be detected using reverse transcription polymerase chain reaction (RT-PCR). The RT-PCR test may be performed in two distinct ways. The cDNA for a two-tube RT-PCR setup is produced in a separate tube before the PCR experiment is run. On the other hand, cDNA is initially synthesized in a first stage of RT-PCR. In a one-tube PCR process, the reverse transcriptase and polymerase are both inactivated at the same time (El-Tholoth et al., 2019). This test is quickly replacing others. When designing an RT-PCR technique, it's crucial to keep in mind a number of critical processes. A first step is the isolation of RNA (Naf'an et al., 2020). Using polymerase enzymes, PCR works on the premise that it can simultaneously amplify and distinguish between many target nucleic acids. However, these methods can often only identify a single kind of virus at a time. Viruses that have multiplied in embryos may be detected in both laboratory and clinical specimens using PCR. It is currently the most reliable method for detecting nucleic acids and has the potential to be very sensitive (Worku et al., 2022). However, the viruses of interest in this research contain RNA as their nucleic acid, thus PCR cannot be used. Since viral RNA serves as a template for reverse transcription, a particular
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Materials and Methods

Viral RNA Extraction

Using Viral Gene-spinTM Viral DNA/RNA Extraction Kit).

Fusion gene sequencing method

F gene confirmation sequencing and phylogenetic tree analyses were carried out using RNA sequencing. We submitted the PCR-positive fusion protein gene to Macrogen Company in Korea to have its RNA sequenced using the AB RNA sequencing technology. Multiple sequence alignment study of the partial fusion protein gene sequence based on Clustal W alignment analysis and the Molecular Evolutionary Genetics Analysis, version 6.0 (Mega 6.0) were used in the DNA sequencing analysis. Maximum Composite Likelihood based upon the UPGMA phylogenetic tree was used to calculate evolutionary distances.

Results

FASTA files of three representative isolates

(Sequenced by Macrogen, Korea)

>AN02
CTCCAGCTTAAGGAGAGCATGGCAGATCGCTGCAACCAAATGAGTCGTGCATGAAGTACCCAGGATTATCACAACCT

>AN03
ATACACCTCATCCAGACAGGATACATCATAGTTAGCCTCCTGGATCTTTTGCAG

>AN07
GTCCGGGATACTCAATTGTTGTTTCCTCTGGCAG

Alignment of the sequences of the three isolated (AN02, AN03 and AN07) against the registered sequences of Newcastle viruses in the database of NCBI were studied, and the result showed that, 94% identity with avian orthoavulavirus 1 isolate ByCk/IR/MAME96/2017, 99% identity with avian orthoavulavirus 1 isolate INDTNC22020 and 86% identity with avian orthoavulavirus 1 isolate INDTNC222020 respectively. These results were shown in Figures (1), (2), and (3).
Figure (1): Avian orthovulavirus 1 isolate ByCk/IR/MAME96/2017 fusion protein gene

Figure (2): Avian orthovulavirus 1 isolate INDTNC222020 fusion protein gene
Based on the phylogenetic analysis performed in this study, rooted phylogenetic tree (Figure 4) of the nucleotide sequences of the fusion protein gene of Newcastle disease virus strains. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 2.20497769 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 61 nucleotide sequences. Evolutionary analyses were conducted in MEGA7. Isolates analyzed in this study are marked with filled circle, whereas those reported earlier from the Iraq are marked with empty circles.

DISCUSSION

NDV outbreaks in Iraq continue to pose a threat to the country's commercial chicken sector despite the widespread use of vaccinations in the country. It has been postulated that environmental factors, such as secondary immunocompromising infections, might impair the efficiency of vaccinations (AlShammari et al., 2020). This could be one explanation for why NDV outbreaks have occurred in populations that have been vaccinated. There is a possibility that vaccination failure and the subsequent epidemic of NDV was caused by inadequate vaccination procedures as well as insufficient levels of flock immunity. Madadgar et al. (2013) proposed three hypotheses to explain the sudden appearance of highly pathogenic NDV. They are as follows: (1) the virus had always been in poultry but was unnoticed until the advent of commercial poultry industries; (2) the virulent virus was enzootic in another species where the less sensitive host revealed the less severe disease; and (3)
the highly pathogenic virus arose from a virus of low virulence by mutation. The first hypothesis proposes that the virus had always been the second theory, on the other hand, has recently emerged as the consensus across communities everywhere. During the panzootic that occurred in the 1970s and 1973, it was discovered that the virus was transferred to certain regions by the migration of captive caged birds, particularly psittacine species, which exhibit some resistance to the viruses that are virulent for chickens. This was discovered during the period of time when the virus was circulating. In spite of the fact that virulent NDV has been discovered in captive caged birds on several times (Madadgar et al., 2013), it has been theorized that this is as a result of interaction with livestock that is sick. In a similar vein, there are few reports of wild bird reservoirs of virulent NDV, with the exception of North American cormorants and perhaps pigeons. These two species are the only two known to harbor the virus. The third hypothesis, which posits that virulent viruses evolve as a consequence of mutation from viruses of intermediate virulence, has been supported by research on the viruses that were responsible for virulent NDV outbreaks in Ireland in 1990. These viruses were shown to have antigenic and genetic characteristics with other NDVs, but they were found to be antigenically and genetically distinct from all other NDVs (Sun et al., 2022). Diagnostic assays based on different areas of the virus have been devised when F gene based diagnostic primers failed to identify NDV (Miller et al., 2010). Last but not least, there is no way to confirm whether or not a flock has been vaccinated since the owner must disclose this information to authorities.

Neighbor-joining was used to estimate the evolutionary history (Saitou and Nei, 1987). The taxonomic lineage is assumed to follow the tree of life derived using the bootstrap method (500 replicates) (Felsenstein, 1985). When a partition is repeated in less than half of the bootstrap replicates, the relevant branch is pruned. In the bootstrap test (500 repetitions), the proportion of trees in which the connected isolates grouped together is shown next to the branches (Felsenstein, 1985). The evolutionary distances are expressed as the average number of base substitutions per site and were calculated using the Jukes-Cantor technique (Jukes and Cantor, 1969). Four different nucleotide sequences were used in the study. The first, second, third, and non-coding locations of the codon table were all included. Blank and empty spots have been filled up completely. The completed dataset had a total of 316 locations. The program MEGA7 was used for the evolutionary analyses (Kumar et al., 2016).

**CONCLUSION**

Virulent strain-specific motifs were found in the majority of circulating strains, as shown by partial sequencing of the F gene cleavage site.

**REFERENCES**


