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# Comparative Biological Pathotyping of Newcastle Disease Virus Sub-genotypes VIIg, VIIh, and VIIi in Specific Pathogen Free (SPF) Chickens

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## ABSTRACT

**Newcastle Disease (ND) is an infectious viral disease in poultry caused by the Newcastle Disease virus (NDV). The NDV that causes ND cases in Indonesia is genotype VII with various sub-genotypes. This study was conducted to compare the *in vivo* pathogenicity of ND virus sub-genotypes VIIg, VIIh, and VIIi in specific pathogen free (SPF) chickens. The virus isolates were isolated and identified as ND virus sub-genotypes VIIg, VIIh, and VIIi. The level of pathogenicity is known by calculating the mean death time (MDT) and the intravenous pathogenicity index (IVPI). The comparations of lesions of NDV were analyzed descriptively and statistically based on clinical signs, the ratio of lymphoid organ weight, and lesions in hematoxylin-eosin (HE). Isolate confirmation was conducted by harvesting the lung organs. ND was detected using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using specific primers of the fusion (F) fragment gene. The sequence was compared with that of other NDVs from GenBank and Analyzed using MEGA-6 software. The analysis results showed that the VIIh and VIIi sub-genotypes were virulent strains due to the MDT <60 hours with IVPI value index of 3.0, the lymphoid organs showing atrophy and lymphocyte depletion. On the other hand, the VIIg sub-genotype was a less virulent strain because it had an IVPI index of 0.0 with MDT >60 hours, and the lymphoid organs showed mild lymphocyte depletion. Based on the in vivo results with the sequence analysis at the cleavage site showed that ND isolates Sub-genotypes VIIh (112RRQRRF117) and GVIIi (112RRQKRF117) were virulent strains, but Sub-genotype VIIg (112GRQGRL117) was less virulent.**

*Keywords: genotype; molecular amplification; Newcastle disease; pathogenicity; tissue tropism*

## INTRODUCTION

One of the challenges in the poultry industry is Newcastle Disease (ND), a viral disease that is highly contagious and infects many species of domestic, exotic, and wild birds (Alexander & Senne, 2008). The ND outbreak was first discovered by Kreneveld in Java, Indonesia, in 1926 and in 1927 by Doyle in Newcastle, Tygne, England, giving the name according to the name of the area (Dzogbema *et al.*, 2021). The virus that causes ND is an important pathogen since it is one of the diseases that have a global economic impact on the poultry farming industry due to the expensive handling, not only losses during an outbreak such as growth disorders, decreased egg production, decreased body weight, and death, but also control measures such as costly vaccination and repeated testing (Pattison *et al.*, 2008).

ND or Avian or Avian orthoavulavirus-1 is a member of the genus Orthoavulavirus of the family Paramyxoviridae in the order Mononegavirales (ICTV, 2020). Newcastle Disease Virus (NDV) is an enveloped RNA virus that is pleomorphic or spherical in shape with a diameter of 100-500 nm (Alexander & Senne, 2008; OIE, 2021). The NDV genome is divided into 2 classes, namely class I which has 9 genotypes and consists of avirulent strains with a size of 1598 nucleotides, and class II which has 18 genotypes consisting of avirulent and virulent strains with a size of 1586 or 1592 nucleotides (Dzogbema *et al.*, 2021). The virulent NDV that causes outbreaks in the world comes from Genotypes V, VI, VII, and IX, and the NDV genotype VII was initially divided into 2 sub-genotypes, namely, genotype VIIa which was spread in Europe and Asia, and genotype VIIb which was spread in South Africa (Aldous *et al.*, 2010), then these 2 sub-genotypes were further classified into several sub-genotypes namely sub-genotypes c, d, e, f, g, h, and i (Miller *et al.*, 2015). Other research states that genotype VII NDV was divided into sub-genotypes VII.1.1, VII.1.2, and VII.2 (Dimitrov *et al.*, 2019). Various studies found that the most common NDV in Indonesia was genotype VII (velogenic) NDV (Pandarangga *et al.*, 2020; Goraichuk *et al.*, 2020). Outbreaks of genotype VII

NDV in Indonesia have been reported in Banjarmasin, Sukorejo, Sragen, and Kudus (Xiao *et al.*, 2012).

In Indonesia, comparative studies of in vivo pathogenicity between sub-genotype have never been carried out. The pathogenicity of NDV can be analyzed through the characterization of the F gene in the cleavage site region, MDT, ICPI, and IVPI (OIE, 2021). Lymphoid organ damage due to the ND virus is associated with immunosuppressive. Knowing the pathogenicity between sub-genotypes, especially on lymphoid organs, is a basis for early selection to become an effective and safe vaccination in the field. This study was conducted to compare in vivo pathogenicity in SPF chickens between NDV sub-genotypes VIIg, VIIh, and VIIi, especially on the lymphoid organs, as an initial test in selecting vaccine seed candidates for the safe vaccine.

## MATERIALS AND METHODS

### Ethical Clearance

This research was approved by the Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta (010/EC-FKH/Int./2022).

### Study Period and Location

This research was conducted from May-October 2022 at Research and Development Department, PT. Sanbio Laboratories, Bogor, West Java, and Pathology Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia.

### Samples

Three NDV viruses (VIIg, VIIh, and VIIi sub-genotype) were isolated from chickens (layers and broilers) from several regions in Indonesia from 2020 to 2021 and have been isolated and identified at PT Sanbio Laboratories, West Java, Indonesia. The information concerning the origin of virus outbreaks, the year of isolation, breed, type, and age of birds involved are presented in Table 1. The ND case on commercial chicken showed clinical symptoms, such as high mortality and morbidity, torticollis, diarrhea, and anorexia. Further research on viral pathogenicity has never been done before.

### Hemagglutination Test

Viruses were used to inoculate 9-11-day-old Specific Pathogen Free (SPF) embryonated eggs for virus isolation via an allantois route, and the NDV was identified through an HA test using standards procedure in OIE (2021).

### Mean Death Time (MDT)

MDT was performed in 9-10-day-old SPF embryonated eggs. Serial tenfold dilutions of allantois fluid (AF) of each virus isolate were prepared, and 0.1 mL of the dilutions ($10^{-6}$, $10^{-7}$, $10^{-8}$, and $10^{-9}$) was inoculated into the allantois cavity using 5 eggs per dilution. The highest dilution at which all embryos died soon was considered as the mean lethal dose (MLD), and the MDT was calculated (FAO, 2002; Nakamura *et al.*, 2014).

### Intravenous Pathogenicity Index (IVPI)

Ten 6-week-old SPF chickens were inoculated intravenously with 0.1 mL of a 1:10 dilution of allantois fluid. The chickens were observed for 10 days after inoculation. The chickens are examined at 24-hr intervals for days and scored at each observation: 0 if normal, 1 if sick, 2 if paralyzed or showing other nervous signs, and 3 if dead (FAO, 2002).

### Collecting the Lymphoid Organs, Hematoxylin Eosin (HE)

Necropsy and pathologic changes in chickens were observed. The tissues of lymphoid organs (thymus, spleen, and bursa of Fabricius) were calculated the ratios, collected, and fixed by 10% NBF were processed and embedded in paraffin. Sections were stained with HE. Data on the ratio of lymphoid organs were analyzed statistically using ANOVA. Histopathological changes were analyzed descriptively.

### Re-Identification Virus

The lungs from infected chickens should be prepared as 20% suspensions in the antibiotic solution using the standard procedure in OIE (2021). The supernatant was followed by RNA extraction. The RNA used for testing was extracted from the samples using TRIzol LS reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. One-step RT-PCR (SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase; Life Technologies, Carlsbad, CA, USA) was used to convert and amplify the extracted RNA samples. After RNA Extraction, the F gene was amplified. The regions of the F gene of NDV isolates were amplified through PCR with one pair of primers (forward: 5'-ATCCAAGCAGGTACCCAACG-3';

Table 1. The location of Newcastle Disease virus outbreaks, year of isolation, type, and age of birds

| Virus | Sub-genotype | Location of outbreak | Year of isolation | Host/Organ isolation | Type of bird | Age |
|---|---|---|---|---|---|---|
| 019/NDV/F/20 | VIIg | Kulonprogo, Yogyakarta | 2020 | Chicken/Lien | Broiler | 11 days |
| 123/NDV/F/20 | VIIh | Palembang, South Sumatera | 2020 | Chicken/Lien | Layer | 34 weeks |
| 183/NDV/F/21 | VIIi | Jember, East Java | 2021 | Chicken/Trachea | Layer | 45 weeks |

reverse: 5'-AAGTCGGAGGATGTTGGCAG-3') (Putri *et al.,* 2021). The PCR process consisted of 1 cycle of 7 min pre-denaturation at 95 °C; 40 cycles of 10-s denaturation at 94 °C; 30-s annealing at 58 °C; extension for 1 min at 72 °C; and a final extension cycle at 94 °C for 10 min. Amplicon PCR was analyzed via gel electrophoresis with 1% agarose (0.5xTris/borate/EDTA) plus 2 μL ethidium bromide and documented visually with a UV transilluminator.

The PCR product was continued with Sanger sequencing. Amplicon PCR was purified with Big Dye Xterminator Purification and Applied Biosystem Big Dye Terminator V3.1 cycle sequencing kit by procedure following the manufacturer's instructions. The sample was put into the well microplate sequencing and read with a Genetic Analyzer 3100 (Applied Biosystems). MEGA6 software is used in sequence data assembly and sequence alignment. The complete F gene sequences of available genotype VII NDV isolates were downloaded from GenBank of the National Center for Biotechnology Information. Phylogenetic analyses were conducted using the Neighbor-Joining method (Tamura *et al.,* 2013).

## RESULTS

### Mean Death Time (MDT) and Intravenous Pathogenicity Index (IVPI)

Isolate sub-genotypes VIIh and VIIi are classified as velogenic strains because they have <60 hours to kill the embryo and cause 100% death in chickens. Isolate subgen VIIg virus is a mesogenic or lentogenic strain because it takes >60 hours and does not cause death in chickens (Table 2).

### Clinical Sign and Collecting the Lymphoid Organs Weight

Isolate sub-genotype VIIg did not show clinical symptoms until the end of the observation. Isolates sub-genotype VIIh had 100% mortality on the 4th and 5th days, while group VIIi had 100% mortality on the 3rd day with the same symptoms, such as green diarrhea, inactive chickens, hair parting, anorexia, and paralysis.

The calculation results of the lymphoid organs ratio weight (thymus, spleen, and bursa of Fabricius) showed that the GVIIh and GVIIi groups showed atrophy. The lymphoid organs of the GVIIg group did not show atrophy (Table 3). A comparison of lymphoid organ weight was analyzed using One Way Anova between the control and treatment groups. The lymphoid organs of sub-genotype VIIg group were not significantly different ($p>0.05$), while the VIIh and VIIi sub-genotype groups were significantly different ($p<0.05$).

### Gross Pathological and Histopathology

The lymphoid organs of sub-genotype VIIh and VIIi groups showed high severity lesions, the sub-genotype VIIg group showed low severity, and no lesions were observed in the control group. Gross pathological and histopathologic lesions in the lymphoid organs are summarized in Table 4, also presented in Figures 1 and 2.

### Re-identification: RT-PCR, Sequencing, Phylogeny, and Genetic Analysis

Molecular NDV identification using the RT-PCR method was based on the amplification of the F gene for NDV screening. Three virus isolates confirmed NDV (Figure 3). The phylogenetic analysis based on the F gene showed that the isolates belong to Genotype VII with sub-genotypes that match the initial virus (Figure 4). Isolate NDV Sub-genotype VIIg contained the amino acid sequences GRQGRL at positions 112-117 at the cleavage site, and sub-genotypes VIIh and VIIi contained RRQR/KRF in the same position.

Table 2. The intravenous pathogenicity index values and the mean death time produced by the Newcastle Disease virus isolates

| Sub-genotype NDV | HA Titer (HAU) | MTD (hours) | IVPI | Pathotype based on MDT | Pathotype based on IVPI | |
|---|---|---|---|---|---|---|
| | | | | FAO, 2002 | FAO, 2002 | Nakamura *et al.,* 2014; Cattoli *et al.*, 2011 |
| VIIg | 9log2 | 74.6 | 0.0 | Mesogenic | Lentogenic | Lentogenic/Mesogenic |
| VIIh | 8log2 | 36.2 | 3.0 | Velogenic | Velogenic | Velogenic |
| VIIi | 8log2 | 50.6 | 3.0 | Velogenic | Velogenic | Velogenic |

Note: NDV= Newcastle Disease Virus; HA= Hemagglutination; HAU= Hemagglutination unit; MDT= mean death time; IVPI= intravenous pathogenicity index.

Table 3. The average ratio of lymphoid organ weight to chicken body weight

| Group | The ratio of the weight of lymphoid organs per chicken weight* | | |
|---|---|---|---|
| | Thymus ± SE** | Spleen ± SE** | Bursa of fabricius ± SE** |
| VIIg | 0.0079 ± 0.00023 | 0.0029 ± 0.00006 | 0.0060 ± 0.00027 |
| VIIh | 0.0028 ± 0.00027 | 0.0022 ± 0.00013 | 0.0024 ± 0.00013 |
| VIIi | 0.0052 ± 0.00057 | 0.0024 ± 0.00014 | 0.0044 ± 0.00034 |
| Control | 0.0085 ± 0.00026 | 0.0029 ± 0.00009 | 0.0067 ± 0.00023 |

Note: *)The ratio of the weight of lymphoid organs per chicken weight (gram). **)The average ratio of the lymphoid organs chicken weight (gram) ± standard error ($p<0.05$).

Table 4. Gross pathological and histological lesion of lymphoid organs

| Organs | Lesions | Group | | | |
|---|---|---|---|---|---|
| | | Control | VIIg | VIIh | VIIi |
| Thymus | HE (necrosis, hemorrhages) | - | + | +++ | +++ |
| | HE (lymphocyte cell depletion) | - | + | +++ | +++ |
| | Atrophy, hemorrhages | - | - | +++ | +++ |
| Lymph | HE (necrosis, congestion) | - | + | +++ | +++ |
| | HE (lymphocyte cell depletion) | - | + | +++ | +++ |
| | Atrophy, congestion | - | + | +++ | +++ |
| Bursa of fabricius | HE (necrosis, lymphocyte cell depletion) | - | + | +++ | +++ |
| | HE (lymphoid gland edema) | - | - | +++ | +++ |
| | Atrophy, Yellowish mucosa | - | - | +++ | +++ |

Note: Lesions - = negative or not found, + = low severity, ++ = moderate severity, +++ = high severity. HE= hematoxylin eosin.

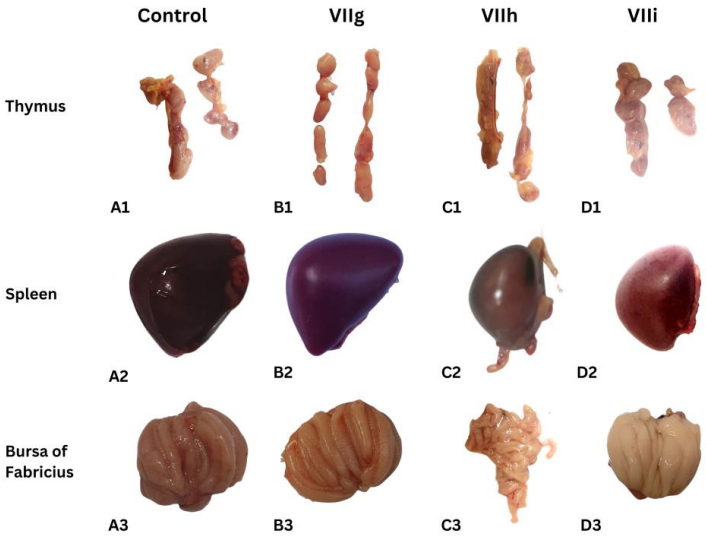


Figure 1. Gross pathological lesions in the lymphoid organs (1) thymus, (2) spleen, and (3) bursa of fabricius. Control (A1, A2, A3) and VIIg (B1, B2, B3) groups showed normal appearance, while for VIIh and VIIi groups, (C1, D1) showed hemorrhage and atrophy, (C2, D2) congestion and atrophy, and (C3, D3) yellowish mucosa and atrophy.

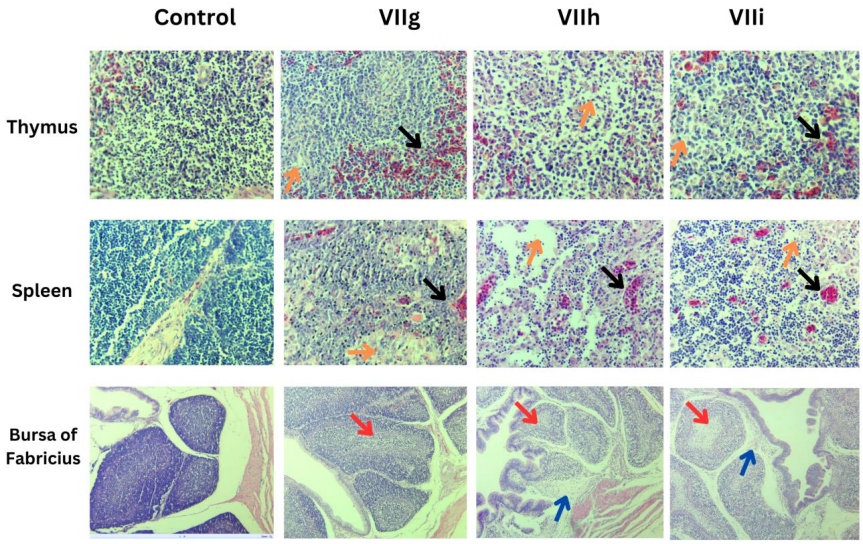


Figure 2. The histopathological lesion in hematoxylin eosin (HE) staining (x400). The lymphoid organs of the control group showed no abnormal lesions. The VIIg group showed hemorrhages (black arrow), lymphocyte cell depletion (yellow arrow) and loss of glandular follicles in the medulla (red arrow) with low severity. The VIIh and VIIi groups showed lymphocyte cell depletion (yellow arrow), necrosis and hemorrhage (black arrow) in the thymus and spleen. Bursa of the Fabricius of VIIh and VIIi showed edema (blue arrow), bursitis, necrosis, and loss of glandular follicles in the medulla (red arrow).

## DISCUSSION

Outbreaks of ND are endemic in various countries, including Indonesia, with discoveries in various regions in Indonesia, such as Yogyakarta, Sulawesi, and Java (Saputri *et al.*, 2021; Goraichuk *et al.*, 2020). Comparative studies of *in vivo* pathogenicity levels between ND sub-genotypes have never been conducted in Indonesia. Viral characterization using the pathogenicity test is essential, as the importance and impact of a given NDV isolate are directly related to its virulence.

### MDT and IVPI

The isolates showed positive results on the HA test (Table 2). HA activity detected in the isolates is signed in the presence of NDV (OIE, 2021). The ability of NDV to agglutinate Red Blood Cells (RBCs) is due to the binding of the HN protein to receptors on the surface of the RBCs (Bilal *et al.*, 2014).

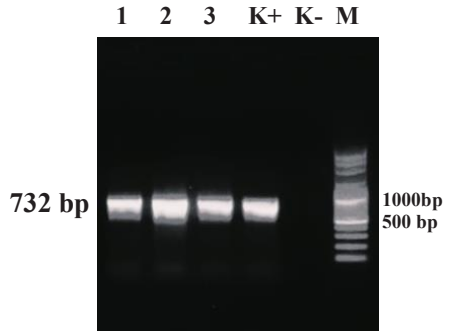


Figure 3. The amplification results of F gene of Newcastle Disease virus. PCR products measuring 732 bp. Amplicons were electrophoresed on 1% agarose gel. Newcastle Disease virus isolates were in lanes 1-3, K+ and K- lines were positive control and negative control, and M was a molecular size marker.

The *in vivo* assessments of virulence are based on MDT and IVPI tests. The chickens inoculated with ND sub-genotype VIIi had an IVPI index of 3.0 and was included as the velogenic strain because they experienced 100% mortality on day 3, while ND sub-genotype VIIh died 100% on days 4 and 5. The time of death of these isolates corresponds to the time of 100% death on days 2 to 5 after inoculation reported by Indriani & Dharmayanti (2016). The chickens inoculated with sub-genotype VIIg until the last observation period had no clinical symptoms and were shown healthy. The IVPI are results according to the MDT result. Sub-genotypes VIIh and VIIi have index IVPI 3.0 with MDT<60 hours categorized as velogenic strain. Genotype VII ND isolates should be categorized as velogenic ND viruses (Rell *et al.*, 2021; Roohani *et al.*, 2015). The IVPI score index of 0.0 and MDT >60 hours in the VIIg sub-genotype group is less virulent and categorized as mesogenic or lentogenic strains (Cattoli *et al.*, 2011; Nakamura *et al.*, 2014). Genotype VII also reported death for more than 60 hours (Eid *et al.*, 2022).

### Clinical Signs and Anatomical Pathology

The deaths of chickens infected with viruses of sub-genotypes VIIh and VIIi following the clinical symptoms reported in the field. Chickens infected with viruses began with a decrease in appetite, causing a decrease in body weight (Ezema *et al.*, 2016), respiratory problems and watery yellow to green feces, feathers parting, becoming inactive, liquid exudated out of the mouth until paralysis occurred before death (Oyebanji


Figure 4. The phylogenetic relationships of the three sample isolates marked with black circles (●) compared to the reference isolates whose sequences have been published in GenBank. The phylogenetic tree was constructed by bootstrap 1000 replications. The three samples were viruses of sub-genotype VIIg (019/NDV/F/20), sub-genotype VIIh (123/NDV/F/20), and sub-genotype VIIi (183/NDV/F/21).

*et al.,* 2017). The VIIg sub-genotype did not experience clinical symptoms until the end of the observation period. This result is somewhat different from the previously published virulence of genotype VII ND (Xiao *et al.,* 2012).

Lymphoid organ atrophy was evident in the ND GVIIh and GVIIi groups. Lymphoid organ atrophy is evidenced by the weight of the lymphoid organs, which is smaller than the control. Descriptively, all treatment groups had a smaller organ weight ratio than the control (Table 3), but when compared statistically, the VIIh and VIIi sub-genotype groups were significantly different from the control, while the VIIg sub-genotype group did not. NDV is an agent that can cause immunosuppression in chickens because it can cause atrophy of the bursa of Fabricius (Cazaban, 2022). NDV can replicate in lymphoid organs such as the spleen, bursa of Fabricius, and thymus (Kang *et al.,* 2016). The thymus, bursa of Fabricius, and the spleen will become atrophy after being infected by Very Virulent Newcastle Disease (VVND) (Ezema *et al.,* 2016; Rehman *et al.,* 2021).

Anatomical pathology in sub-genotype VIIh and VIIi groups found in organs is under that reported by Nooruzzaman *et al.* (2022) in which chickens infected with the velogenic strain ND virus were found to have hemorrhage, congestion, and atrophy in the lymphoid organs (Figure 1). The spleen, thymus, and bursa of Fabricius are lymphoid organs in poultry and function as lymphocyte producers and maturation. Ezema *et al.* (2016) reported that the spleen, thymus, and bursa of fabric atrophied after being infected with the ND virus. In contrast to what was reported by Etriwati *et al.* (2017), where the lymphoid organs swelled but were similarly found necrotic, hyperemic, and congested due to NDV.

## Histopathology

Histopathological changes in lymphoid organs were found in accordance with several previous reports (Figure 2). The thymus was found to have necrosis and showed depletion of lymphocytes, the bursa of Fabricius showed severe necrosis, lymphocyte depletion, hyperemia, follicular atrophy, folded bursal epithelium and cysts formation, and the spleen was congested and showed depletion of lymphoid cells (Courtney *et al.,* 2013; Ezema *et al.,* 2016; Etriwati *et al.,* 2017). Lymphocyte depletion in the cortex and medulla is a pathological change found in the thymus of chickens infected with ND starting from the third day after infection (Mohammadamin & Qubih, 2011). The presence of NDV in lymphoid tissue can cause lymphocytic in the middle of the germinal center and produce nuclear debris resulting in necrosis and depletion of lymphocytes in the spleen, while in the bursa of Fabricius is depleted, which is characterized by a reduction in the number of lymphocyte cells in lymphoid follicles thus the follicles appeared empty (Kabiraj *et al.,* 2020). NDV infection induced the increased apoptosis of B cells in the bursa of Fabricius, causing a decrease in humoral immunity and high oxidative stress causing damage to the bursa of Fabricius (Teo *et al.,* 2017). As a result of the infection of the NDV, causing lesions in the lymphoreticular organs, indicate that the ability to fight infection is impaired (Etriwati *et al.,* 2017). The virulent NDV activated the innate immune system and disrupted the metabolism causing pathological changes in the immune system (Cheng *et al.,* 2022). Current velogenic strain NDV infection also caused pathological changes in lymphoid organs such as the thymus, spleen, and bursa of Fabricius (Nooruzzaman *et al.,* 2022).

## Re-identification

The results of virus reidentification remained the same, namely, genotype VII ND virus. From the phylogenetic analysis, it can be ascertained that the ND viruses used were indeed viruses of sub-genotype VIIg (019/NDV/F/20), sub-genotype VIIh (123/NDV/F/2020), and sub-genotype VIIi (183/NDV/F/21) (Figure 4). The level of pathogenicity of the ND virus *in vivo* test results can be compared with the results of sequencing. The level of pathogenicity can be determined based on molecular analysis of the gene sequences encoding the F protein, where the F protein has sections called cleavage sites which are areas that greatly influence the process of viral infection since they determined viral virulence (Rell *et al.,* 2021). In the cleavage site, the fusion protein (F) has amino acid characteristics for virulence in chickens (OIE, 2021). Molecular pathotyping was carried out based on the sequence of proteolytic amino acids in the cleavage sites (residue numbers 112-117) of the NDV isolate strain. This method is very fast and reliable for NDV pathotypes compared to MDT, IVPI, or ICPI (Battisti *et al.,* 2013). Virulent NDVs have amino acid F (phenylalanine) at residue 117, while viruses with low virulence have amino acid L (leucine) in the same region (Pattison *et al.,* 2008). The results showed that the isolates of the VIIh and VIIi sub-genotypes had the 112RRQR/KRF117 motif, which is characteristic of velogenic strains and has the same sequence as the previous NDV which was isolated in 2010 in Indonesia (Xiao *et al.,* 2012). NDV isolate sub-genotype VIIg (019/NDV/F/20) has a motif of 112GRQGRL117, which is a pathotype of a less virulent ND strain.

The molecular characteristics found were under the results of *in ovo*, *in vivo*, and histopathological changes found in the organs of chickens infected with the virus. PCR results for reidentification of lung organ samples in all treatment groups showed positive results for ND according to the results of the hemagglutination test. A positive isolate hemagglutination test indicates the presence of the ND virus (OIE, 2021). Chicken lung organs have been detected with ND virus starting 24 hours after infection (Kabiraj *et al.,* 2020). ND viruses genotype VIIh and VIIi are velogenic because they have amino acids phenylalanine in the cleavage site, causing egg embryo death in less than 60 hours and an IVPI index of 3.0 (Pattison *et al.,* 2008; FAO, 2002; Nakamura *et al.,* 2014; Cattoli *et al.,* 2011). The velogenic nature of the ND virus genotypes VIIh and VIIi corresponds to the appearance of clinical symptoms of infected chickens starting on the 3$^{rd}$ day and death on the 4$^{th}$ day after infection (Nooruzzaman *et al.,* 2022). Velogenic ND virus infection causes obvious changes in the lymphoid

organs characterized by atrophy and lymphoid depletion. Based on the report of Rabiei *et al.* (2021), the NDV that caused the outbreak, especially in Indonesia, was NDV genotype VII, which had shifted in tropism from gastrointestinal or respiratory to a lymphotropic virus that attacks lymphoid organs, including the spleen and bursa of Fabricius, causing lymphoid depletion.

The sequencing results of the cleavage site of virus VIIg number 117 found leucine amino acid, making it less virulent (Pattison *et al.,* 2008). The molecular characteristics of the VIIg virus are under the results of *in ovo* and *in vivo* tests. The mesogenic ND virus causes embryonic death within 60-90 hours with an IVPI index of 0.0 (FAO, 2002; Nakamura *et al.,* 2014; Cattoli *et al.,* 2011). ND VIIg virus did not cause clinical symptoms or death in chickens. Histopathological lesions of the ND virus are less virulent and lighter than virulent ND virus in lymphoid organs (Hussein *et al.,* 2018).

## CONCLUSION

Based on molecular characterization, MDT, IVPI, and histopathological observation, the ND virus sub-genotype VIIg is a mesogenic NDV strain, while the ND isolates of sub-genotypes VIIh and VIIi are velogenic NDV strains. Isolate sub-genotype VIIg was the least virulent, which did not cause death in chickens, but low severity lymphocyte depletion was found. The NDV sub-genotypes VIIi and VIIh were highly virulent that made the lymphoid organs as well as the occurrence of atrophy and severe depletion of the lymphoid organs.

## CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this article.

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